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E. R. LING

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Dairy chemistry:...
C.F.T.R.I.

A TEXTBOOK OF DAIRY CHEMISTRY

Volume One

THEORETICAL

A TEXTBOOK OF DAIRY CHEMISTRY

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By

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PREFACE TO THE SECOND EDITION

During the years which have elapsed since the publication of the first edition of this text-book, dairying has developed into a highly technical industry. This evolutionary process continues, and it is desirable that the type of training should keep pace with or even anticipate the changing conditions of the industry. These developments are reflected in the new edition which has been completely rewritten and enlarged. The author has presented information which he considers to be of fundamental importance and has not allowed any existing examination syllabus to dominate the selection of subject-matter or its method of presentation. Nevertheless, it is hoped that the second edition will prove a valuable asset to those reading for College Diplomas or University Degrees and to all engaged in the technical branches of the dairy industry. Copious references to original papers will be found throughout the text: the student is strongly advised to read some of them, for this first-hand information will stimulate an interest which can seldom arise out of a text-book treatment. These references are also an expression of the author's indebtedness to the many workers who have advanced the science of dairying.

To those with a good knowledge of chemistry and physics the various formulæ and equations will prove useful, but it has been the endeavour of the author to treat the subject-matter in such a manner as to enable the salient facts to be appreciated by those with only an elementary knowledge of these two subjects.

Owing to the increased scope of the new edition, it has been found more convenient to issue the practical section as a separate volume.

EDGAR R. LING

Loughborough
1943

PREFACE TO THE FIRST EDITION

In the preparation of this text-book of Dairy Chemistry, the author has in mind the needs of students of Agriculture and Dairying, and the subject-matter is based largely upon a course of lectures delivered by the author at the Midland Agricultural College. Those wishing to keep abreast of the progress of Dairy Science are so often impressed with the broad aspects of the subject, touching as it does the specialised sciences of chemistry, pure and applied, physics, bacteriology, medicine, and agriculture, the publications of which have to be searched for relevant information. As a consequence, the student so often finds that owing perhaps to lack of adequate library facilities or the short duration of the course, dairy chemistry is apt to become one of the most difficult sections of their curriculum.

The text of the book has been drawn from diverse sources, and in the references quoted the author pays tribute to those who have contributed to the rapid progress of dairy science, and at the same time hopes that the references may be of use to those who seek a more specialised knowledge of some aspects of the work. The student is urged to read at least two of the original papers quoted, for in so doing an interest will be stimulated which can never be derived solely from a text-book treatment.

Although written on the assumption that the readers will already possess some knowledge of chemistry and physics, it is hoped that under the guidance of a tutor, the book may prove helpful to those attending certificate courses at Agricultural Institutes, to whom this assumption may not apply.

EDGAR R. LING

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CHAPTER I

THE CONSTITUENTS OF MILK

It requires only a superficial examination to establish the fact that milk is a mixture, yet this is a matter of fundamental importance. Indeed it may be considered the mixture *par excellence*. Raw milk is unique amongst mixtures in so far as when kept at ordinary temperatures, it separates into three obvious constituents. The rising of fat globules to form the cream layer and the separation of curds and whey in sour milk are very familiar changes which have found their way into early writings of every country. In his treatise *The Haven of Health*, Thomas Cogen in 1636 stated: "Milk notwithstanding that it seemeth to be wholly of one substance, yet it is compact or made of three severall substances, that is to say in effect of Creame, Whey and Curds." Then follow some observations on the manifold virtues of milk, particularly "for them which be oppressed with melancholy, which is a common calamity to students." In modern, less picturesque language, milk is described as a mixture of three main constituents—Water, Fat, and Non-fatty Solids—the latter generally named Solids-not-Fat (S.n.F.), which term notwithstanding its affront to the English language has proved inviolate to any attempts at improvement.

Further examination of the fat and non-fatty solids resolves them into several constituents as shown in the table on page 3. This process of resolving the milk constituents into their simpler components is still in progress. For instance, the true fat is known to be a mixture, but the precise number and constitution of its components are not yet fully established. Casein, one of the milk proteins, is believed by some workers to be a mixture and it is hoped that further research will establish the identity of these fractions. It is a matter of importance that the student should realise that the scientific study of milk is by no means complete and that important additions to our knowledge are being made year by year. The necessity for frequent consultation of current literature is thus obvious.

Some of the constituents are present in exceedingly minute quantities, but they cannot be ignored on that account. For

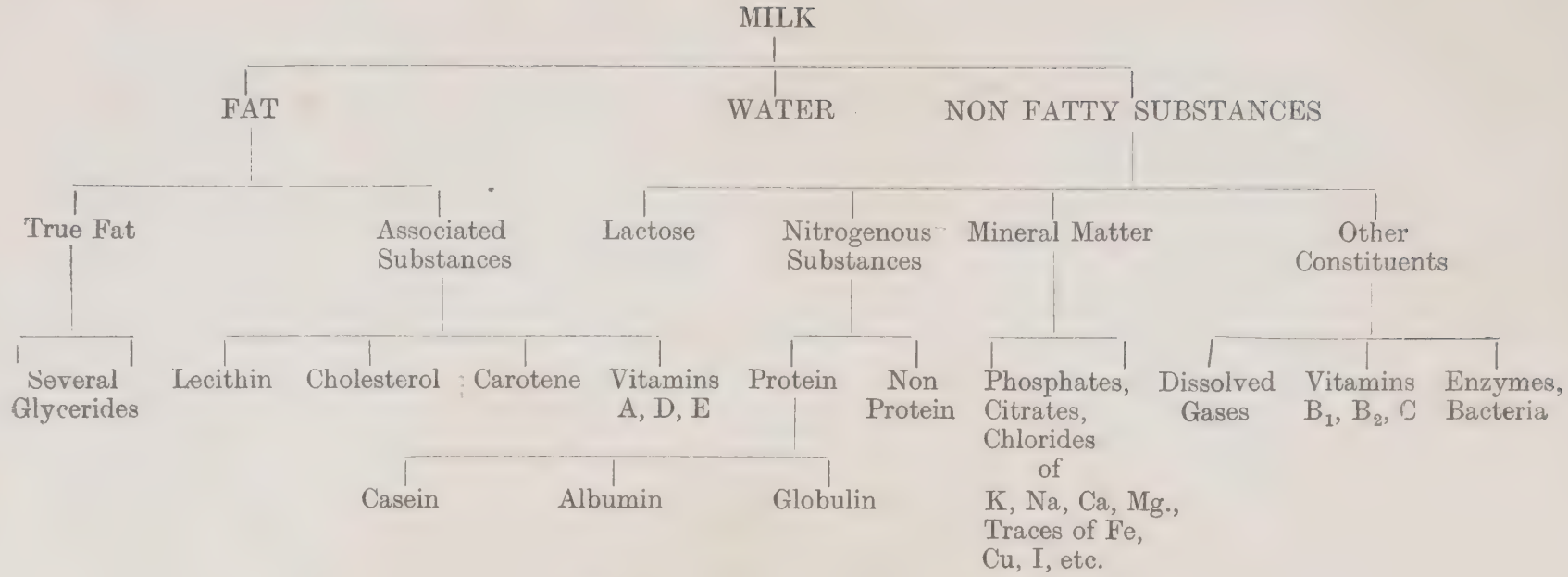
example, although vitamins, lecithin, and dissolved gases are present in very small quantities they are each centres of chemical changes which on occasion dominate the properties of the major constituents. Their significance will be manifest in later chapters.

Arising from the conception of milk as a mixture is the fact that the proportions of its constituents may show considerable variation. This being a salient feature of all mixtures, one should not expect milk to be rigidly constant in composition. Although this may seem a very elementary fact, it is nevertheless one which is frequently overlooked.

Each of the milk constituents will be studied in detail. Of necessity, they must be considered separately, but it is known that in milk they do not enjoy such independence. The protein casein is linked with some of the mineral constituents, some of the water is bound by proteins; while the fat is intimately associated with a number of other substances. These are a few examples of the interdependence of the milk constituents, which, taken separately, are analogous to the various building-stones of an edifice the plans of which are not completely in our possession.

There is a further consideration. Besides being a mixture in the sense that it contains a number of separate chemical compounds, milk is also a physical mixture. Fat is present in the form of an emulsion; the proteins and some mineral matter are in the colloidal state; and lactose together with the remainder of the mineral matter are in true solution. Three distinct physical states are coexistent and to some extent are in a state of poise. This applies more particularly to the equilibrium between the colloids and the true solution. Chemical changes in milk and dairy products have their effect on these physical states and, moreover, changes in physical condition of some of the constituents may modify their normal chemical behaviour. These observations are amply justified in the study of the fat of milk, cream or butter.

Lactose.—Lactose or milk sugar, which appears to be formed exclusively in the milk of mammals, is present in cow's milk to the extent of about 4·7 per cent. Human milk contains about 6·3 per cent, so that in modifying cow's milk for infant feeding, it becomes necessary to add lactose. It is one of the few substances in true solution in milk, a subject which will receive greater attention under a different heading (page 66). On crystallisation from water it forms hard, gritty crystals,


MILK
FAT
WATER
NON FATTY SUBSTANCES
True Fat
Associated Substances
Lactose
Nitrogenous Substances
Mineral Matter
Other Constituents
Several Glycerides
Lecithin
Cholesterol
Carotene
Vitamins A, D, E
Protein
Non Protein
Phosphates, Citrates, Chlorides of K, Na, Ca, Mg., Traces of Fe, Cu, I, etc.
Dissolved Gases
Vitamins B_1, B_2, C
Enzymes, Bacteria
Casein
Albumin
Globulin

$C_{12}H_{22}O_{11}.H_2O$. These crystals appear in some milk products under interesting circumstances. Although present in sweetened condensed milk, they are normally far too small to be detected by the tongue. If, however, the conditions of manufacture permit the growth of larger crystals, the product will assume a gritty texture. The crystals may also appear in ice cream when the mix contains too high a proportion of milk solids. Some peculiar properties of dried milk are due to the union of anhydrous lactose, $C_{12}H_{22}O_{11}$, with water to form the hydrate, $C_{12}H_{22}O_{11}.H_2O$. Lactose is only faintly sweet, being about one-sixth the sweetness of cane sugar, and it therefore imparts merely a faint sweet taste to milk. It is interesting to note that although containing about the same concentration of lactose, sweet (non-acid) whey is much sweeter than milk, due no doubt to the removal of casein, which masks the flavour.

Lactose belongs to the di-saccharose group of carbohydrates and is composed of one molecule each of glucose and galactose united, with the elimination of a molecule of water in such a manner as to leave only one reactive aldehyde group in the lactose molecule. By the action of the enzyme lactase, which is present in the secretion of the intestinal glands, lactose undergoes hydrolysis into its two components glucose and galactose.

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Galactose}}{C_6H_{12}O_6}$$

Dilute mineral acids will also bring about this change. There is still some difference of opinion regarding the presence of lactase in milk, but if present at all, it has no great practical significance. Certain varieties of yeast such as those present in Kephir cultures are able to carry out the initial hydrolysis and to proceed with the alcoholic fermentation of the two monosaccharose sugars. This fermented milk contains small amounts of alcohol (approximately 0·7 per cent), carbon dioxide, together with lactic acid, and is occasionally prepared for special dietaries. It is claimed that the casein of this product is in a very digestible form.

Aqueous solutions of lactose reduce Fehling's solution and ammoniacal silver nitrate solution, but after hydrolysis the reducing power is doubled owing to the production of two active reducing groups. Although insoluble in alcohol or ether, it readily dissolves in hot acetic acid. Under slightly acid conditions lactose may be oxidised to formic and lævulinic acids (see page 105),

whereas oxidation with concentrated nitric acid yields oxalic and carbonic acids.

When heated between 110–130°C., lactose hydrate crystals lose their water of crystallisation. Above 150°C. they turn yellow, while at 175°C. they brown considerably, forming lacto caramel. These temperatures are interesting in view of the general belief that the slight browning and characteristic cooked flavour of heated milk are due to the formation of caramel from milk sugar. No milk or milk product would be processed at temperatures high enough to caramelise pure lactose. This apparent anomaly compels us to recognise that the properties of the individual milk constituents may be different from their behaviour in milk where they are in contact with so many other compounds. Thus it is possible for lactose as it exists in milk to caramelise at much lower temperatures. Again, it is known that when lactose is gently warmed with nitrogenous bases like ammonia, certain amines, or amino acids, any of which could be present in heated milk, brown coloured complexes are formed. Naturally, the extent of these changes will depend upon the severity of heating and the time of holding. While pasteurised milk may be but slightly affected, the changes are much more apparent in sterilised and evaporated milk.

Commercially, lactose is a very valuable product, being used in the preparation of infant and invalid foods. Its high nutritional value is well recognised. As it is to human beings and most animals the only source of the sugar galactose, which is present in brain and nerve tissues, Gortner[1] deprecates the substitution of cane sugar for lactose in the preparation of infant foods. It is believed that lactose aids the assimilation of calcium and phosphates from the intestines, but this may well be part of the 'tonic' effect of lactose on the alimentary canal. The chief source of lactose is whey, and the method of preparation will be described in a later chapter.

Lactic fermentation.—Lactose is an easy prey for various micro-organisms which find their way into milk, even under the most careful methods of production and handling. Under favourable conditions of temperature, the faintly sweet taste of raw milk gradually changes to a more stringent flavour and there is a simultaneous production of the characteristic odour of souring milk. The progress of the change may be demonstrated by

[1] R. A. Gortner, *Outlines of Biochemistry*, 1938, p. 655.

incubating a bottle of raw milk at 37°C. Acidity tests are made at regular intervals and the results plotted on a graph. The points lie on a smooth curve from which it is seen that at first, the development of acidity is slow, this period being followed by one of vigorous development until the final stage is reached, showing a marked retardation at an acidity corresponding with about 1 per cent lactic acid. At an acidity of 0·3 to 0·4 per cent it is possible to detect a sour taste, while a sample of milk with an acidity of 0·6 to 0·7 per cent will curdle at ordinary temperatures. The exact time taken for these changes to occur will depend upon the number and nature of the micro-organisms present and the temperature of storing. It may be a matter of days or merely a few hours.

These changes are in the main due to the bacterial conversion of lactose to lactic acid.

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$$

$$2C_6H_{12}O_6 = 4\underset{\text{Lactic acid}}{CH_3CHOH\ COOH}$$

With the usual mixed bacterial population of raw milk, there is a check to further acid production at approximately 1 per cent lactic acid, not because there is no more lactose left, but because this concentration is unfavourable to further activity. Neutralisation of milk when it has reached this stage results in a renewed burst of activity with the production of more acid. A glance at the equations will show that 1 per cent lactic acid is formed from approximately 1 per cent of lactose, so that some 75 per cent of the initial lactose content is still available when this retarding effect is established. Pure cultures of different bacteria show pronounced differences in their toleration of accumulations of acid.

Lactic acid in its pure form has neither the odour nor the flavour of sour milk. Like most biological reactions, the souring of milk cannot be represented by any one equation. There are numerous by-products, including carbon dioxide gas, acetyl-methyl-carbinol, di-acetyl, and butyric acid, the two latter possessing powerful odours. The main reaction is certainly as shown in the equation, but the nature and quantity of the by-products is determined by the types of bacteria and conditions of storage of the sample. This explains the care with which cream-starters are propagated and stored. Only the correct cultures will produce the required flavours and aroma in quantities sufficient to give butter its desired characteristics. It is considered that of every

100 parts of lactose attacked during the souring of milk some 95 pass into lactic acid, while the remainder are converted into the various by-products.

When under control, these changes are of great value in the making of butter and cheese, but otherwise they cause severe losses to the dairy industry and considerable annoyance to the housewife. By modern methods of clean milk production which aim at reducing bacterial contamination at the source and by keeping the milk below 50°F., or at as low a temperature as possible, such wastage may be avoided. Although at such temperatures bacterial action is very considerably retarded it does not entirely cease; and it therefore becomes necessary to exercise care in the bacteriological cleanliness of storage vessels even when the milk is kept cold.

Both casein and mineral matter are affected by the development of lactic acid. The former is normally united with calcium, but as the concentration of lactic acid increases, more and more of the calcium is withdrawn from casein to form calcium lactate. Further, some of the colloidal calcium phosphate is converted to soluble calcium hydrogen phosphate. Thus the development of lactic acid alters the physical equilibrium between the true solution and the colloidal state. The former increases at the expense of the latter, and the net result is an increase of osmotic pressure and a corresponding decrease in the freezing-point of the sample. This effect on the freezing-point assumes great practical significance in the detection of added water by the cryoscopic method (page 99).

Lactic acid is a valuable commercial product and may be produced by controlled fermentation of lactose in whey. The initial product is calcium lactate, from which may be prepared pure lactic acid, metallic lactates or lactic esters. These substances are used in a variety of industries, including Plastics and Resins, Textiles, Fine Chemicals, etc., for which whey may be regarded as a possible raw material. Further, as lactic acid may be fermented to butyric acid according to the following equation, it offers another possible utilisation of surplus whey.

$$\underset{\text{Lactic acid}}{2CH_3CHOH\,COOH} = \underset{\text{Butyric acid}}{C_3H_7COOH} + 2CO_2 + 2H_2$$

Milk Fat.—Microscopic examination of milk reveals the presence of fat globules whose diameters vary extensively on either side of the average, 0·003 mm. These dimensions will be better appreciated

from a calculation by P. F. Sharp,[1] who finds that the fat globules of a quart of Guernsey milk if laid end to end would form chain 5,860 miles in length. By the same calculation, the total surface area of these globules covers 1,050 square feet. In any one sample of milk there is a great diversity of globule size, but there is a definite increase in the proportion of smaller globules with advancing lactation. There are also differences between the breeds. Generally, those giving milk of greatest fat content also give more of the larger globules, while those giving least fat are characterised by a much larger proportion of smaller globules.

Provided it has not been subjected to violent agitation, a sample of cooled raw milk will show the fat globules in clusters of many hundreds of individuals. This tendency to cluster is a factor of great importance in the separation of cream by centrifugal force or by the force of gravity. (Page 123.) In cream, the clusters are closely packed, but neither in milk nor in cream does the fat run together although the globules are actually in contact with their immediate neighbours. It would appear, therefore, that each globule enjoys an independent existence through the good offices of a protective layer. This layer is either 'tacky' by nature or may be rendered so by some other milk constituent or by suitable physical conditions.

Much work has been done in an attempt to elucidate the nature of this protective film. Although we have advanced a long way from the earliest theory that each globule is enclosed in an enveloping membrane, there are still many gaps in our knowledge. Reference was made to the very large surface area of the globules. Such large areas associated with small particles create considerable surface forces which are responsible for the phenomenon of adsorption. This phenomenon is common to all systems in which small particles of matter are in contact with liquids or gases, the main proviso being that they should be small enough to present a great surface area per unit weight. This condition is fulfilled by particles of colloid size such as casein in milk, and the fat globules. Thus the modern view is that these globules concentrate at their surfaces certain milk constituents which, forming a very thin film, are able to prevent their merging into a continuous fat phase.

Examination of this protective layer has shown it to contain a variety of substances such as—protein, phospho-lipid (page 20).

[1] P. F. Sharp, *J. Dairy Sci.*, 1940, 23, 771.

phosphatase, an unidentified ether soluble substance, and a complex of riboflavin—phosphoric acid—protein, which is known to possess the properties of the Schardinger enzyme (i.e. it stimulates the simultaneous reduction of methylene blue and the oxidation of formalin when both of these are added to milk). However, the physical condition of the fat determines to some extent the precise composition of the protective layer, and in the case of cream will modify its physical properties very considerably (page 126). It is through the peculiar properties of the adsorbed layer that when metals like copper are dissolved in minute quantity by milk, they are able to stimulate oxidative changes in the fat phase. The dissolved metal forms a metallic proteinate by direct union with the globule surface protein, or it unites with the plasma proteins and is then adsorbed by the globules. In either case the water soluble metal is brought into direct contact with the fat which otherwise would remain inaccessible. In this respect the globule surface material functions in a manner far from protective. Palmer and his associates have published a number of papers[1] showing that it is possible to 're-surface' the fat globules of milk and cream, but by so doing the fat is more vulnerable to attack by various agencies leading to changes which will be discussed under the heading 'fat deterioration.' The modern view is thus far removed from the conception of the fat globule neatly wrapped in an inert membrane. *Indeed, the adsorbed layer is at times the centre of vigorous chemical action.*

The physical properties of pure fat extracted from milk, cream, or butter, establish it to be a mixture. Its colour varies considerably; it melts over a range of about 5°C. and sets over a similar range. Moreover, these two ranges do not coincide. Hilditch[2] gives 28–33°C. and 24–19°C. as the melting and setting points. If the fat were just one compound, it would melt at one definite temperature and solidify at the same point. Taken singly, some of the compounds present in milk fat would melt at approximately 65°C., while others would be liquid even at 0°C. There are many compounds which normally comprise milk fat and each has its own melting-point and other distinctive physical properties. On warming the solid mixture it is the constituents of lowest melting-point which first liquefy. Some of the remaining solids will dissolve in the liquid and will thus liquefy at a

[1] Palmer *et al.*, *J. Dairy Sci.*, 1935, 18, 827 ; 1939, 22, 543; 1940, 23, 861.
[2] T P. Hilditch, *The Industrial Chemistry of the Fats and Waxes*, 1941, p. 134.

temperature well below their normal melting-points. As the temperature is raised, more and more of these solids will dissolve until finally the whole of the fat is in the liquid condition. The temperature at which this occurs is not strictly the melting-point, but rather the temperature at which the least soluble fat fractions will dissolve in the liquid obtained at lower temperatures. Similarly, the setting-point is really the temperature at which the various fractions crystallise from the mixed solution. There is invariably a time and temperature lag in this crystallisation, so that setting-points do not correspond exactly with the 'melting'-points. This circumstance is accentuated in milk and cream owing to the dispersal of the fat into innumerable individual globules. If the fat were in a continuous phase, the first crystals would encourage others to appear and the setting would take place more rapidly and over a smaller range of temperature. This, however, is not the case with milk and the consequence is that several hours must elapse before the fat globules are adjusted to alterations in temperature. The specific gravity of milk warmed to room temperature is higher than that of the same sample cooled to the same temperature after warming above 37°C. Naturally, these two values move nearer to the true value if the samples are kept for some time at room temperature. This time lag is estimated by Sharp[1] to occupy at least four hours, and is a matter of importance in the study of cream and in milk analysis, where the specific gravity is sometimes used for rapid estimations of non-fatty solids. Further evidence of the heterogeneous nature of milk fat is furnished by variations in its specific gravity, 0·936–0·946 at 15°C., and refractive index, 1·459–1·462 at 15°C. It is soluble in the fat solvents petroleum and ether, and although only slightly soluble in ethyl alcohol it is readily soluble in hot amyl alcohol. Water is unable to dissolve the fat, but fat is able to dissolve very small amounts of water.

Reference has been made to the physical properties of milk fat being determined by the nature and proportions of its constituents. Chemically, it consists of a mixture of true facts (i.e. glycerides of fatty acids) which hold in solution or by adsorption, a number of substances, including lecithin, cholesterol, carotene and fat-soluble vitamins.

The True Fats.—Glycerol, being a tri-hydroxy alcohol, unites with three molecules of the same (or different) fatty acids

[1] P. F. Sharp, *J. Dairy Sci.*, 1940, 23, 771.

producing fat, which may be resolved into its components by hydrolysis, according to the following equation :

$$\begin{array}{l} CH_2 - OOC.C_{17}H_{35} \\ | \\ CH - OOC.C_{17}H_{35} + 3H_2O \\ | \\ CH_2 - OOC.C_{17}H_{35} \\ \text{Stearin} \end{array} = \begin{array}{l} CH_2OH \\ | \\ CH\ OH \\ | \\ CH_2OH \\ \text{Glycerol} \end{array} + 3C_{17}H_{35}COOH \quad \text{Stearic acid}$$

The enzyme lipase is the natural hydrolytic agent. In the laboratory the decomposition of fat may be effected by treatment with superheated steam, or more conveniently with alcoholic sodium hydroxide, whereby it is saponified to the sodium salts of the constituent fatty acids (soaps) and glycerol. This solution, on acidification with sulphuric acid, liberates the fatty acids.

$$\begin{array}{l} CH_2 - OOC.C_{17}H_{35} \\ | \\ CH - OOC.C_{17}H_{35} + 3NaOH \\ | \\ CH_2 - OOC.C_{17}H_{35} \\ \text{Stearin} \end{array} = \begin{array}{l} CH_2OH \\ | \\ CH\ OH \\ | \\ CH_2OH \\ \text{Glycerol} \end{array} + 3C_{17}H_{35}COONa \quad \text{Sodium stearate}$$

$$2C_{17}H_{35}COONa + H_2SO_4 = \underset{\text{Stearic acid}}{2C_{17}H_{35}COOH} + Na_2SO_4$$

While all the true fats, being similarly constituted, will react in this manner, they differ in the number and relative proportions of fatty acids united with glycerol. If milk fat were treated in the same way, it would furnish a mixture of at least sixteen fatty acids, some soluble in water, but most of them insoluble and therefore appearing as a white precipitate. These acids have been the subject of much research because the physical and chemical properties of fats are so largely dominated by their constituent fatty acids. So much emphasis is laid upon them in technical literature that the unwary student might easily fall into the error of regarding fat as a mere mixture of fatty acids. The differences between some of these acids are to be seen in the Table. Although most of them are odourless and almost tasteless, some, and more particularly butyric acid, possess a powerful odour and may impart a bitter taste to dairy products. The liberation of butyric acid will be discussed under the heading 'fat rancidity.'

Component Fatty Acids of Milk Fat.

Formula	Name	Approx. % by weight	At ordinary temperatures	Volatility in steam	Water Solubility
A. Saturated compounds of the type $C_nH_{2n+1}COOH$					
C_3H_7COOH	Butyric	3·5	Liquid	Volatile	Soluble
$C_5H_{11}COOH$	Caproic	2·0	Liquid	Volatile	Slightly soluble
$C_7H_{15}COOH$	Caprylic	1·0	Liquid	Volatile	Very slightly soluble
$C_9H_{19}COOH$	Capric	2·0	Solid	Volatile	Very slightly soluble
$C_{11}H_{23}COOH$	Lauric	2·5	Solid	Slightly volatile	Almost insoluble
$C_{13}H_{27}COOH$	Myristic	10·0	Solid	Very slightly volatile	Insoluble
$C_{15}H_{31}COOH$	Palmitic	25·0	Solid	Non-volatile	Insoluble
$C_{17}H_{35}COOH$	Stearic	10·5	Solid	Non-volatile	Almost insoluble
$C_{19}H_{39}COOH$	Arachidic	0·5	Solid	Non-volatile	Insoluble
B. Unsaturated compounds with one double bond. $C_nH_{2n-1}COOH$ type					
$C_9H_{17}COOH$	Decenoic	5·0	Liquid	Non-volatile	Insoluble
$C_{11}H_{21}COOH$	Dodecenoic				
$C_{13}H_{25}COOH$	Tetra Decenoic				
$C_{17}H_{33}COOH$	Oleic	33·0	Liquid	Non-volatile	Insoluble
C. Unsaturated acids of the $C_nH_{2n-3}COOH$ type. (Two double bonds)					
$C_{17}H_{31}COOH$	Linoleic	4·0	Liquid	Non-volatile	Insoluble
D. Other more unsaturated acids containing 20 to 22 carbon atoms					
—	—	1·0	Liquid	Non-volatile	Insoluble

NOTES.—In giving the above proportions of the fatty acids, it is well recognised that various factors may cause variation in the composition of milk fat. These will be discussed in a later chapter. In spite of these variations the balance of steam volatile non-volatile acids, saturated/unsaturated acids, etc., renders mild fat unique and thus serves to distinguish it from animal or vegetable fats.

It will be seen that, in common with many other natural fats, Oleic and Palmitic acids are in greatest quantity, representing one-third and one-quarter of the total respectively. Then follow Stearic and Myristic acids, each representing approximately one-tenth of the total. It is the minor components, however, which distinguish milk fat from others. Another important consideration is that some 43 per cent of the total acids are unsaturated. This alone would account for milk fat being chemically active, given the right conditions.

If each separate acid formed one compound with glycerol, there would be about 16 individuals in milk fat. There is ample evidence to show that the glycerides actually present in milk fat are compounds of glycerol with two or even three different fatty acids. Thus the total number of possible compounds is very large indeed. Arup[1] resolved butter fat into solid and liquid fractions at various temperatures and analysed each fraction. He found that the volatile acids were present in solid and liquid fractions, although of course the latter contained more than the former. Butyric acid united to glycerol would give liquid tributyrin, but as this acid is found in the solid fractions, it must also be united to glycerol along with other solid acids. The problem of discovering the precise constitution of these molecules is obviously very complex, but the evidence to date allows the following conclusions to be drawn.[2]

1. The acid in greatest quantity, Oleic, is mainly distributed between four types of glycerides.

A	B	C	D
CH_2 (Oleic)	CH_2 (Oleic)	CH_2 (Oleic)	CH_2 (Oleic)
|	|	|	|
CH (Palmitic)	CH (Palmitic)	CH (Oleic)	CH (Stearic)
|	|	|	|
CH_2 (R)	CH_2 (Stearic)	CH_2 (Palmitic)	CH_2 (R)

where R denotes one of the lower fatty acids. Of these four, group A is the most common.

2. A number of molecules are fully saturated.

3. The Palmitic radical is present in some 75 per cent of the molecules.

As the actual percentage of each acid is subject to some variation, one must expect their distribution in the various glycerides to show similar variation. Arising out of the extensive research on the chemical constitution of the glycerides, the

[1] *Analyst*, 1928, 53, 641. [2] Hilditch, p. 170.

modern theory of milk fat formation was conceived. The forme theory that the phospholipids of the blood were the precursor of milk fat is no longer accepted, for reasons which need not b gone into at this juncture. The simpler theory that the mammary gland elaborates milk glycerides from the oleo-glycerides of th blood is more in keeping with the facts at present to hand.

Chemical Changes which may occur in Milk Fat.

A. *Hydrolytic Rancidity.*—Reference has been made to th hydrolysis of fat by the enzyme Lipase. This change, whic results in the liberation of free fatty acids, is the cause of th taint commonly known as rancidity. It will be understood tha the change is *never complete*, one reason being that an accumula tion of water-soluble butyric acid inhibits further action of th enzyme. However, butyric acid has so powerful an odour tha the reaction need only proceed to a very slight degree for pronounced rancid odour and flavour to become evident.

The occurrence and behaviour of the lipases of milk and dairy products have formed the subject of many researches from whic a number of facts of great interest have emerged. Unfortunately some of these are apparently contradictory and it is still im possible to connect all the observations in any satisfactory theory One very potent disturbing factor is the secretion of lipases by micro-organisms and the consequent lipolysis being governed by different conditions from those pertaining to the natural lipas of milk. Another source of confusion has arisen through th measurement of either the water-soluble fatty acids or the fa acidity as being criteria of lipolysis, whereas since the reactio liberates both, a complete picture is possible only when both serum and fat acidity are measured.

The salient observations are given below.

1. Lipolysis may occur at relatively low temperatures (i.e. nea the freezing-point of water). Milk or cream in the natural stat usually has a negative temperature coefficient, by which is mean that if lipolysis occurs, it is more extensive at lower than a higher temperatures.

2. It is significant that when steps are taken to suppress micro organisms, the natural lipase functions with a positive tempera ture coefficient.[1] [2] In the opinion of Fouts,[1] lipolysis of raw

[1] E. L. Fouts, *J. Dairy Sci.*, 1940, 23, 245.
[2] Rohen and Sommer, *J. Dairy Sci.*, 1940, 23, 831.

cream is normally dominated by the micro-organic factor rather than the natural lipase.

3. The extent of lipolysis in milk stored at 5°C. (41°F.) for 48 hours is greatly reduced by rapid cooling to this temperature.[1]

4. Inactivation of lipase by heat is first apparent when raw cream is heated to 110°F. and held there for 150 minutes. At 140°F. momentary heating reduces lipolysis by one half, while holding for 35 minutes causes complete inactivation. Momentary heating at 155°F. also prevents lipolysis.[2]

5. Lipolysis varies considerably in the milk of different cows, and is apparently unrelated to breed, season, or yield. Although there have been instances of cows in advanced lactation giving milk of bitter flavour due to lipolysis, there is as yet no clear relationship between stage of lactation and lipase activity. In this respect, the feeding of the cow is a disturbing factor as it has been observed that green foods tend to inhibit lipase action in late lactation milk.[3]

6. One effect of the liberation of fatty acids is a reduction of surface tension causing increased foaming and, with cream, difficult churning properties.

7. Under normal conditions there is little lipolysis of raw milk owing to the protective action of the adsorbed globule surface.[4]

8. The physical condition of the fat and the nature of the globule surface so largely control lipolysis that it is difficult to discriminate between *lipase activity* and *surface permeability*.

9. Lipolysis is encouraged by homogenisation agitation, particularly when the globules are liquid or semi-liquid, or warming pre-cooled samples to 30°C. (86°F.) and cooling to 10°C. (50°F.) or below.[5] In all these activating processes the normal globule surfacing is altered.

10. Homogenisation of butter fat in separated milk produces globules with a newly absorbed surface which does not afford the same protection as the original material. Such reconstituted samples show greatly increased lipolysis with a positive temperature coefficient.[6]

11. Lipolysis is adversely affected by oxidation, particularly when dissolved oxygen is accompanied by traces of copper or

[1] Krukovsky and Herrington, *J. Dairy Sci.*, 1942, 25, 241. [2] Ibid., 231.
[3] Tarassuk and Regan, *J. Dairy Sci.*, 1943, 26, 987.
[4] Krukovsky and Sharp, *J. Dairy Sci.*, 1938, 21, 671.
[5] Herrington and Krukovsky, *J. Dairy Sci.*, 1939, 22, 137.
[6] Tarassuk and Richardson, *J. Dairy Sci.*, 1941, 24, 667.

other heavy metals.[1] Lactic acidity is also stated to be a restraining influence.[2]

Another type of rancidity known as KETONIC Rancidity is caused by certain types of moulds growing on the surface of butter. In this case the lower fatty acids are broken down to ketones of the type $C_5H_{11}CO.CH_3$ or $C_9H_{19}CO.CH_3$, which possess a heavy odour.

B. *Fat Oxidation producing an Oxidised Taint* (*Oxidative Rancidity*).—In dealing with the composition of milk fat, reference was made to the important fact that over 40 per cent of the fatty acids are unsaturated. These unsaturated molecules are the centres of oxidative reactions resulting in the production of taints which have been described as "oily," "cardboard," "metallic," or "tallowy," according to their intensity. Since they are the result of oxidation they are more correctly described as 'oxidised' taints. There is no doubt that the double bonds of the unsaturated acids are the centres of these changes. After a period of induction of varying duration the fat absorbs oxygen to produce peroxide compounds which may rupture at the peroxide linkage to give aldehydic substances, or which as suggested by Ellis[3] may undergo molecular rearrangement to give hydroxy ketones.

$$\cdot CH:CH\cdot + O_2 = \cdot CH(-O-)\cdot CH\cdot = 2\cdot CH:O$$

$$\text{or} = \cdot C(OH) : C(OH)\cdot = \cdot CH(OH)CO\cdot$$

The peculiar flavours and odours of oxidative taints may be caused by the peroxides or such decomposition products as epihydrinaldehyde, which is the substance reacting with phloroglucinol in the Kreis test for oxidised fat. Hilditch[4] suggests that this substance may be derived from linoleic acid by the following reactions.

$$R_1CH:CH.CH_2.CH:CHR_2 + 2O_2 = R_1CH(-O-)CH.CH_2.CH(-O-)CH.R_2$$

$$= R_1CHO + R_2CHO + CHO.CH_2CHO.$$

$$CHO.CH_2.CHO = CH(OH):CH.CHO = CH_2 . CH(-O-) . CHO$$

Epihydrin aldehyde

[1] W. L. Davies, *J. Dairy Res.*, 1932, 3, 254; Krukovsky and Sharp, *J. Dairy Sci.*, 1940, 23, 1119.
[2] W. L. Davies, *Chemistry of Milk*, 2nd edit., p. 81.
[3] Ellis, *J. Soc. Chem. Ind.*, 1926, 45, 193.
[4] Hilditch, *The Industrial Chemistry of the Fats and Waxes*, 1941, 325.

The presence of peroxide compounds may be detected by their ability to liberate iodine from acid potassium iodide solutions, by which reaction also they may be estimated.

Fat oxidation differs from lipolysis not only in the chemical reactions involved, but in the various conditions governing the change. Oxygen, whether atmospheric or in the dissolved condition, is obviously an essential requirement. Light exercises a stimulating effect, particularly in the ultra-violet wavelengths. One very common cause of the development of an oxidised taint in milk is its exposure to direct sunlight, whereby the dissolved oxygen is rendered much more active. The quantity of dissolved oxygen in milk may be considerably reduced by bacterial growth. This explains an apparent anomaly that the taint seldom develops in milk of poor hygienic quality. Several workers have observed the more frequent occurrence of the taint in winter than in summer, and in milk kept in cold store. There is no doubt that conditions calculated to conserve the dissolved oxygen of milk are favourable to the oxidative reactions. It is estimated that if milk could be de-aerated to the level of 0·4–0·5 mg. oxygen per litre there would be no fat oxidation or loss of ascorbic acid (vitamin C).[1] Exposure to sunlight is known to cause a rapid removal of dissolved oxygen due to its activation and absorption by the unsaturated fats. There is a considerable weight of evidence to show that traces of the heavy metals, copper in particular, exercise a powerful catalytic effect on these changes. This metal sometimes finds its way into milk through the use of worn coolers in which the tinned surface is no longer entire. Milk is thus in contact with tin and copper at the same time and as a consequence copper dissolves under the influence of the potential difference thus created.[2] No doubt the presence of protein also favours solution of the copper through the formation of copper proteinates, which become adsorbed by the fat globules and in this manner the metal is brought into contact with the fat. Iron, cobalt, nickel, chromium, and manganese are also effective, but to a much lesser degree than copper. Tin and aluminium have little effect. Copper is a normal milk constituent, being present to the extent of 0·12 parts per million, but if the concentration rises to 1·5 p.p.m. an oily taint may result. Cream, butter, and milk

[1] P. F. Sharp *et al. Ass. Bull* (Int. Ass. Milk Dealers), 1942, 34, 365.
[2] Mattick, *J. Agr. Sci.*, 1927, 17, 388.

powders are similarly sensitive to the catalytic effect of copper, and the subject will be discussed again under the appropriate headings.

Fat acidity, whether by lipolysis or by fat absorption of lactic acid is also considered to be favourable to these oxidative reactions. Although the centre of all these changes is undoubtedly the double bond of unsaturated fatty acids, it must not be assumed that the saturated fats are incapable of oxidation. Indeed, Hilditch[1] considers that under the influence of peroxidase they may produce ketones, but whether such is possible under conditions normally prevailing in milk is a matter of some doubt.

It is also important to recognise that lecithin contains unsaturated fatty acid molecules which are susceptible to oxidation. Several workers consider that the oily taint originates in lecithin,[2 3] which is not only absorbed on the fat globules, but which is also present in the aqueous phase. Such claims are strengthened by the observation that separated milk has been known to develop the taint; and by measurement of the Iodine value of the fat of milk during the progress of oxidation. Since the absorption of iodine is a measure of the degree of unsaturation it is only reasonable to expect the Iodine value of a fat to decrease as the double bonds are broken. Several researches into this aspect of the problem failed to establish this anticipation. It is very significant that Nelson and Dahle[4] found that although the Iodine value of the fat showed no substantial change during the development of an oily taint, there were marked decreases in these values for the phospho-lipid (lecithin-containing) fraction.

The present position, therefore, is that while all are agreed that the taint originates from the double bonds of unsaturated fatty acids, there are some who insist that only the true fat is involved, whereas others maintain that lecithin is the reacting substance. As the oily taint is observed at an early stage in the oxidative process, *it may well be that lecithin is responsible for its initial development and that being absorbed on the surface of the fat globules it is able to stimulate similar reactions within the fat itself. The powerful tallowy taint of advanced oxidation would thus represent*

[1] Hilditch, *The Industrial Chemistry of the Fats and Waxes*, 1941, 333.
[2] Dahle and Palmer, *Bull. Penn. Agric. Exp. Sta.*, 1937, No. 347.
[3] Swanson and Sommer, *J. Dairy Sci.*, 1940, 23, 201.
[4] Nelson and Dahle, 1940, *J. Dairy Sci.*, 23, 391.

the climax of a chain of reactions which commence with the attack on lecithin.

There are many biological reactions which demonstrate the existence of inhibitory substances, and fat oxidation is one of them. Of recent years a great deal of attention has been devoted to substances which prevent or delay fat oxidation. They are usually designated Anti-oxidants, and appear to act by virtue of the fact that they are able to break the chain of reactions which occur during fat oxidation.[1] In so doing they are themselves oxidised whereby their anti-oxidative powers are removed. This is what is happening during the induction period, the duration of which depends on the nature and quantity of anti-oxidants. Thus, they cannot be considered as negative catalysts owing to their inability to survive the reaction which they influence.

Some of the known anti-oxidants are cereal flours and their extracts, cereal germ oils, Vitamin E, hydroquinone, and substances of the poly-hydroxy-benzene type, carotene, ascorbic acid (Vitamin C), lipase, pancreatic enzymes, sulphites, potassium-iodide, hydrogen-sulphide and its derivatives. Some are specific in their action, protecting one kind of fat only. Some are normal milk constituents, namely, carotene, ascorbic acid, lipase, and hydrogen-sulphide derivatives which may be formed in heated milk. Reference has been made to the more frequent occurrence of an oily taint in milk during the winter months, and although conservation of dissolved oxygen may be an important factor it is the view of some investigators that the winter ration with its lower green food supply is an equally important factor. Much of the evidence is conflicting, but in general leads to the conclusion that a ration which provides an adequate supply of good quality green foods is most likely to furnish milk rich in carotene and ascorbic acid and thereby protected in some measure against oxidation. It would also appear that the anti-oxidant properties of carotene rich milk are due not so much to carotene but to some other compound closely associated with it. The formation of sulphydryl compounds by decomposition of serum or fat globule proteins is considered by Gould[2] to explain his observation that milk heated to 80°C. did not develop an oxidised taint. Corbett and Tracy consider that the greater resistance to

[1] Mattill, 1931, *J. Biol. Chem.*, 90, 141.
[2] Gould, 1939, *J. Dairy Sci.*, 22, 1017.

oxidation shown by condensed milk may also be explained on similar lines.[1] Attempts have been made to prevent oxidation by de-aeration or the addition to milk of anti-oxidants, in particular oat flour extracts (avenised milk), and they have met with varying measures of success.

The study of the protective nature of ascorbic acid has furnished valuable information regarding the mechanism of the development of the oxidised taint in copper-contaminated milk. Olson and Brown[2] found that while high concentrations of ascorbic acid were protective, lower concentrations actually caused the taint to develop in copper-contaminated samples. As it is an established fact that hydrogen peroxide appears during the copper-induced oxidation of ascorbic acid, the authors considered that the former substance may produce the taint. This view was confirmed by the observation that additionof 0·1 per cent hydrogen peroxide to copper-contaminated samples caused the taint to appear.

The mechanism of the development of copper-induced oxidised taints was formulated as follows :

1. Reduction of cupric copper to the cuprous form and simultaneous oxidation of ascorbic acid.

2. Oxidation of cuprous copper by atmospheric oxygen and the simultaneous appearance of hydrogen peroxide.

3. Oxidation of the phospholipids by hydrogen peroxide to give an oxidised taint.

This cycle of changes is inhibited by potassium iodide in concentrations greater than 0·1 per cent.

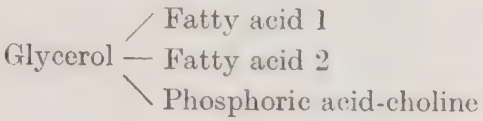
As the tri-peptide-glutathione is able to enter the same cycle of reactions the authors conclude that this substance may also be involved in the development of copper-induced oxidative taints in milk. Other active oxidation-reduction substances such as thiamin, pyridoxine, riboflavin, which are normal milk constituents, did not enter into these reactions.

Substances Associated with Milk Fat.

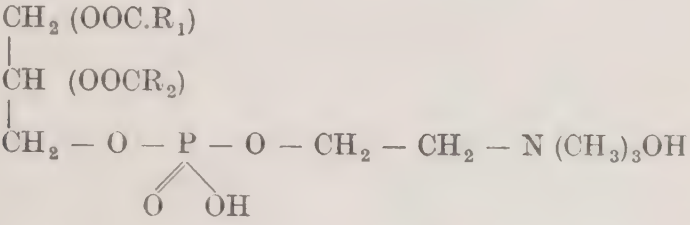
Lecithin.—This compound is a member of a most important group known as the Phospholipids (i.e. phosphoric acid compounds possessing a fatty nature). Their biological significance will be appreciated from the fact that they occur in all living cells, in particular nerve and brain tissues and egg yolk. Cell protoplasm

[1] Corbett and Tracy, 1940, *J. Dairy Sci.*, 23, 209.
[2] Olson and Brown, 1942, *J. Dairy Sci.*, 25, 1027, 1041.

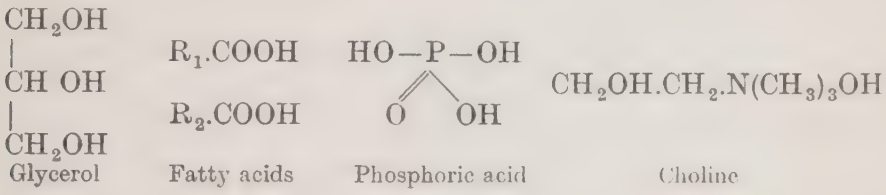
is a complex union of phospholipids, sterols, and protein. The most common phospholipids are compounds of glycerol, fatty acids, and phosphoric acid, the latter being also attached to a nitrogenous base. Of the fatty acids, both saturated and unsaturated types are present, while of the nitrogenous bases, choline is a common member. Lecithin may be written :



or more fully :



On complete hydrolysis it is broken down to produce its four constituents : glycerol, fatty acids, phosphoric acid, and choline.



The phospholipids possess some exceptional properties. Although fatty by nature and soluble in fat and the fat solvents, they are intensely hydrophilic (i.e. they have a strong affinity for water). When in contact with water they imbibe large quantities and swell in a peculiar budding manner. Lecithin and allied compounds in milk and cream thus form a link between the fat and aqueous phases. As the compound contains a basic nitrogenous group in addition to one free acid group of the phosphoric acid, the substance is able to combine with both acids and bases.

The phospholipids of milk are of three types: lecithin, cephalin, and sphingomyelin in the approximate ratio of 9 : 5 : 1.[1] Both lecithin and sphingomyelin contain the choline radical, but the latter also possesses another nitrogenous base. Cephalin differs from lecithin in what instead of choline as the nitrogenous base,

[1] Holm, Wright, and Deysher, 1936. *J. Dairy Sci.*, 19. 631.

amino-ethyl-alcohol, $CH_2OH.CH_2NH_2$, is united to the phosphoric acid. Although these and other differences exist, it will be convenient to refer to all the milk phospholipids under the single heading 'lecithin', particularly as it constitutes some 60 per cent of the total. Being fat soluble and at the same time hydrophilic they are excellent emulsifying agents, and no doubt serve to stabilise the milk fat emulsion. By the formation of lecitho-protein complexes they also serve to bring metals into contact with milk fat and thus initiate the process of fat oxidation. Their intense water-binding capacity assumes considerable importance in the moisture relations of dried milk. The quantity present in milk is small, yet their reactivity and outstanding versatility of physical properties make them important constituents. It will be observed from the Table that lecithin is evenly distributed between both fat and aqueous phases.

At one time it was considered that milk fat was produced from blood phospholipids, but this view is no longer held.[1] Formerly lecithin was considered to be rather heat-sensitive, but by eliminating the possibility of bacterial decomposition, Perlman[2] has shown this view to be incorrect.

LECITHIN CONTENT OF MILK AND SOME MILK PRODUCTS

	Heinemann[3]	Rewald[4]	Holm *et al.*[5]
	Per cent	Per cent	Per cent
Milk . .	0·035 — 0·036	0·0314	0·0337
Separated milk	0·015 — 0·018	—	0·0169
Cream (41 per cent fat)	—	—	0·1816
Buttermilk .	0·114 — 0·126	—	0·1872
Butter . .	0·153 — 0·212	—	0·1819
Whole milk powder .	—	0·24	—

An interesting observation is made by Thurston and Barnhart[6] to the effect that lecithin is partly responsible for the rich flavour

[1] Graham *et al.*, 1936 *Proc. Roy. Soc. B.*, 120, 330.
[2] Perlman, 1935, *J. Dairy Sci.*, 18, 113, 125.
[3] Heinemann, 1939, *J. Dairy Sci.*, 22, 707.
[4] Rewald, 1937, *Le Lait*, 17, 225.
[5] Holm, Wright, and Deysher, 1936, *J. Dairy Sci.*, 19, 631.
[6] Thurston and Barnhart, 1935, *J. Dairy Sci.*, 18, 131.

of buttermilk and other dairy products. Much attention has been focused on the constituent fatty acids of lecithin. In marked contrast to milk fat there are no acids lower than myristic, the saturated fatty acids of the $C_nH_{2n+1}COOH$ type being represented by myristic, palmitic, stearic, and arachidic acids. Oleic acid predominates over the four or five unsaturated acids present.[1] It is obvious that since lecithin has provision for only two fatty acid radicals there must be a number of these compounds, just as there are many individual glycerides in milk fat.

Hydrolysis of lecithin may take place at any of the glyceryl-ester bonds and at the choline-phosphoric acid link and may be brought about by lipases (or more correctly lecithinases). None of the products of hydrolysis would impart a taint, but if oxidation occurs two taints are possible. Attack on the double bonds of the constituent unsaturated fatty acids may result in the development of an oily taint, and oxidation of choline may produce a fishy taint. As trimethylamine $(CH_3)_3N$ is known to possess a very powerful odour, it was naturally assumed to be the cause of such fishy taints. There are several objections to this assumption, one being that the slightly acid reaction of milk is not conducive to the existence of the free base. There is evidence to show that the causative substance is trimethylamine oxide which unites with unsaturated fatty acids to produce compounds of a fishy nature.[2] Indeed it is considered that such substances are more characteristically fishy than trimethylamine itself.

Being an oxidative reaction, it is to be expected that the factors known to favour the development of an oily taint will also stimulate fishiness. Studies of butter and milk powders, the two dairy products which are susceptible to this taint, confirm this view, and again contamination with traces of copper is known to be a most powerful stimulant. As in the case of the oily taint all the factors are cumulative. Further reference to the subject will be made under the appropriate headings.

Fishiness in milk may arise occasionally from the feeding of sugar-beet by-products such as molassed beet pulp. In this case the trimethylamine oxide is derived from betaine, a normal constituent of the sugar-beet. As the base diffuses into the milk from the blood, it is clear that one way of avoiding this particular

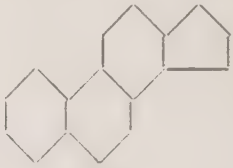
[1] Hilditch and Maddison, 1941, *Biochem. J.*, 35, 24.
[2] W. L. Davies, 1936, *Agric. Progress*, 13, 112.

taint is to feed the pulp either during or immediately after milking so as to allow sufficient time for the trimethylamine oxide to be excreted before the next milking. Although the problem formed an interesting research it is felt that further reference would be irrelevant and those interested should consult the original paper.[1]

Cholesterol.—($C_{27}H_{45}OH$.) This compound is a member of an important group of substances known as Sterols (i.e. solid alcohols). These, like the phospholipids, are widely distributed in animal and plant cells. They are found in the bile, in brain and nerve tissues, in blood and egg yolk. In chemical constitution they are closely related to the sex hormones, vitamin D, and the saponins. They contain the phenanthrene nucleus (1) attached to a cyclopentene ring (2) forming the structural foundation represented by formula 3.

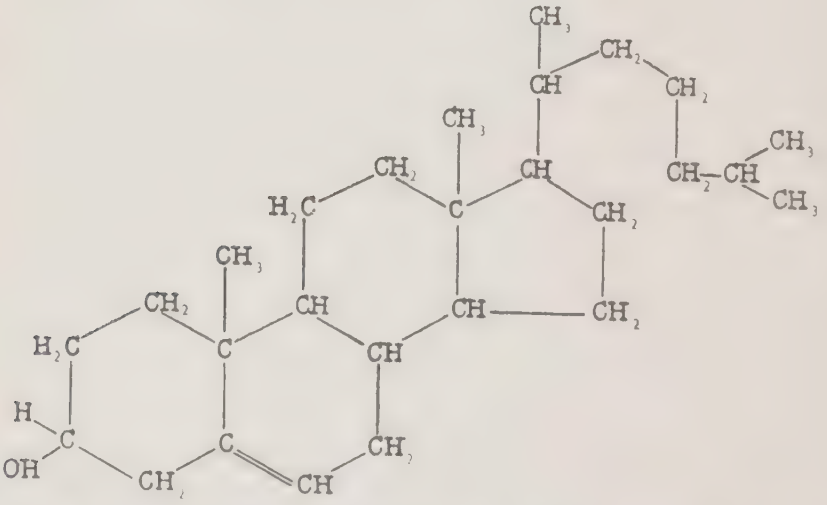
(1)

(2)



(3)

Cholesterol possesses the formula:



While the general configuration of the sterols is the same, they differ in the number and position of their double bonds. They are, in fact, outstanding examples of the manner in which slight alteration of the ornamentation around the original design may

[1] Davies, 1936, *J. Dairy Sci.*, 7, 14.

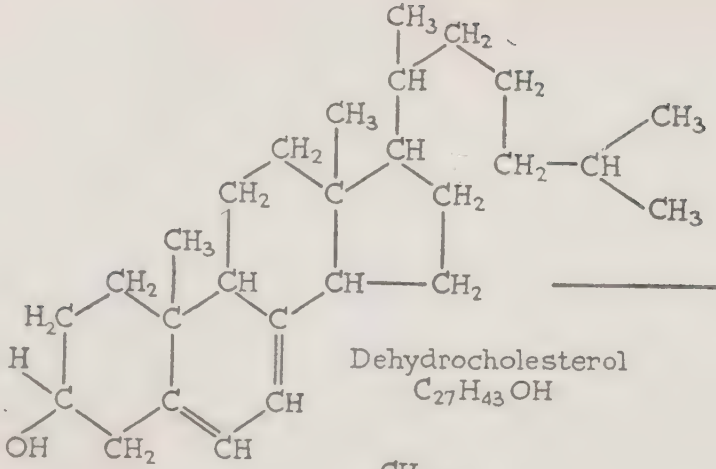
lead to pronounced physiological differences. Thus while cholesterol is absorbed from the digestive tract, the vegetable sterols are not. Again, ergosterol, the sterol present in yeast possesses no vitaminic properties, but exposure to ultra-violet light causes a rearrangement of the molecule in which the second ring of the phenanthrene foundation is opened and an extra double bond is created. The resulting compound possesses strong anti-rachitic properties and is, in fact, one of the vitamins D (calciferol). In spite of their complicated structure they are essentially unsaturated monohydroxy alcohols capable of forming esters with acids. Saponification of fats does not affect the sterols they contain, so that extraction of the residue with ether furnishes a means of isolating the sterol.

The difference in physical properties between cholesterol and the vegetable sterols is used in the detection of vegetable fats in butter, for although the ration of the cow contains vegetable sterols, they do not pass into the milk. There are a number of these vegetable sterols but they are usually called Phytosterol, using this as a family name just as the name lecithin is used to denote all the phospholipids in milk.

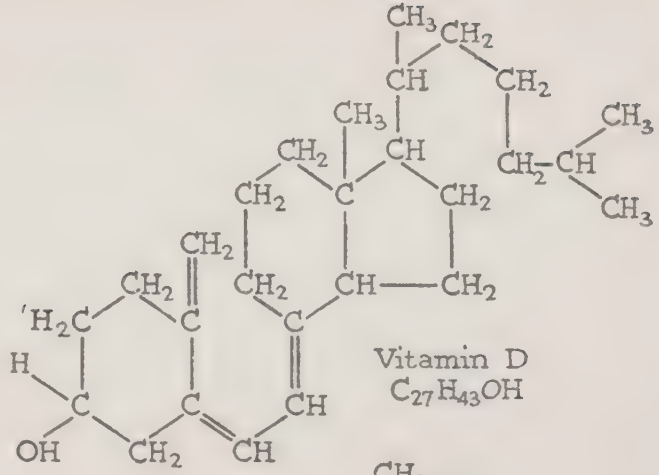
Although cholesterol is insoluble in water, the presence of lecithin is known to assist solution. The close association of these two substances is interesting because cholesterol is able to exercise some control over the intense hydrophilic nature of lecithin. R. A. Gortner[1] states that a retarding or even inhibitory effect on lipase action is exerted by cholesterol. It may be that all these effects are closely related and may account for some of the peculiarities of lipolysis.

Milk fat contains about 0·32 per cent of cholesterol, corresponding to 0·01 per cent in milk of 3·2 per cent fat. Together with lecithin it undoubtedly plays a part in maintaining the stability of the fat emulsion. There is ample evidence to show that more than one sterol is present. Exposure of milk to ultra-violet rays increases its anti-rachitic properties, and in view of the closely related structure of cholesterol and vitamin D, it was natural to assume that the effect was due to a transformation of the former into the latter. Careful experiments with pure cholesterol exposed to ultra-violet light failed to produce vitamin D, and it is thus clear that milk cholesterol contains one or more substances which are precursors of vitamin D.

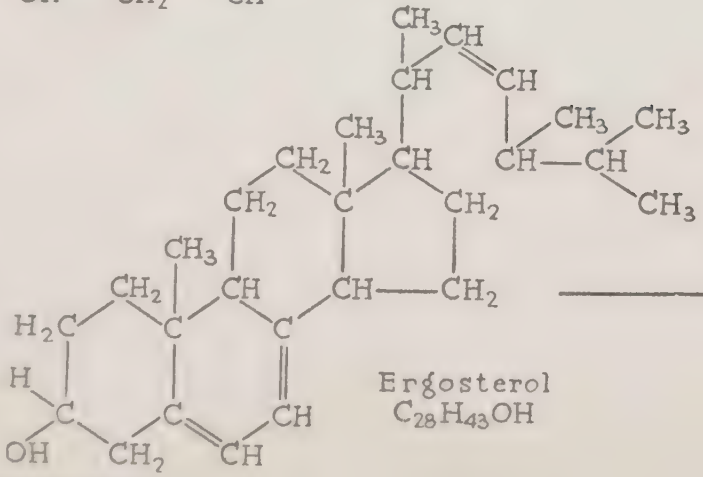
[1] R. A. Gortner, 1938, *Outlines of Biochemistry*, p. 800.



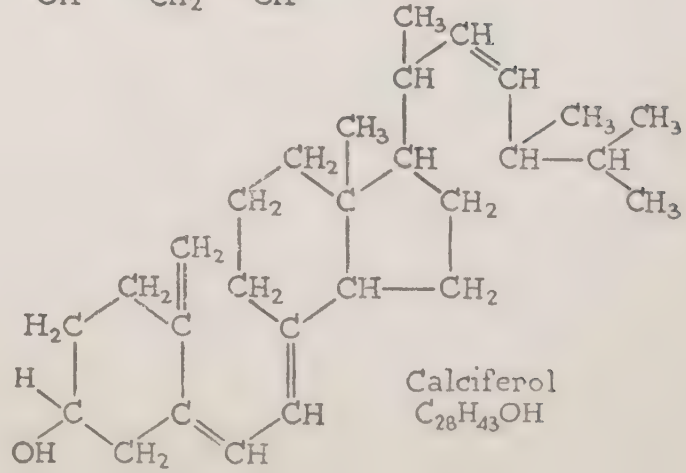
Dehydrocholesterol
$C_{27}H_{43}OH$



Vitamin D
$C_{27}H_{43}OH$



Ergosterol
$C_{28}H_{43}OH$



Calciferol
$C_{28}H_{43}OH$

It is probable that dehydro-cholesterol, $C_{27}H_{43}OH$, is present, which on irradiation is transformed into vitamin D. Similarly, ergosterol may also be present, $C_{28}H_{43}OH$, which passes into calciferol by the same process of molecular rearrangement. These transformations are shown in the formulæ given on p. 26.

Carotene.—The rich colour of cream, characteristic of summer milk, is due to the fat soluble pigment carotene. The depth of colour varies very considerably according to the nature of the food consumed by the cow. From the common observation that summer cream is much richer in colour than that produced in the winter months comes the natural conclusion that the supply of green foods is the main factor concerned in maintaining the carotene content of milk fat.

Carotene is one of the pigments of the green leaf and is also present in the colouring matter of carrots. While the pure substance possesses a red-brown colour, in very dilute fat solutions it imparts a rich yellow colour. Being insoluble in water it is able to colour only the fat phase of milk and the richer colours of cream and butter are due to the higher fat contents of these products. Like lecithin and cholesterol it is but one member of an important family of compounds. The carotenoids, which have a wide distribution in the vegetable kingdom include carotene and xanthophyll, which are the yellow pigments of green leaves. Annatto is an extract of bixin present in the seeds of *Bixin orellana*, while other common members of the family are lycopene, the red pigment of tomatoes, and zeaxanthin, of yellow maize.

The empirical formula of carotene is $C_{40}H_{56}$. In structure it consists of two six-membered carbon rings connected by a long hydrocarbon chain, $C_{22}H_{26}$, in which there are nine double bonds. Each terminal ring contains a double bond and it is evident that the compound is highly unsaturated.

The structural formula reveals the fact that the compound is symmetrical either side of the position marked by the arrows, and that there are many possibilities of isomerism. The formula shown is that of β carotene. Gortner[1] points out that a similarity exists between the structure of carotene and that of phytol, which is a component of the chlorophyll molecule. It is very suggestive that this green pigment which is the active agent in photosynthesis should possess a chemical structure which would allow

[1] R. A. Gortner, 1938, *Outlines of Biochemistry*, p. 740.

of the formation of carotene, the physiological importance of which is well recognised.

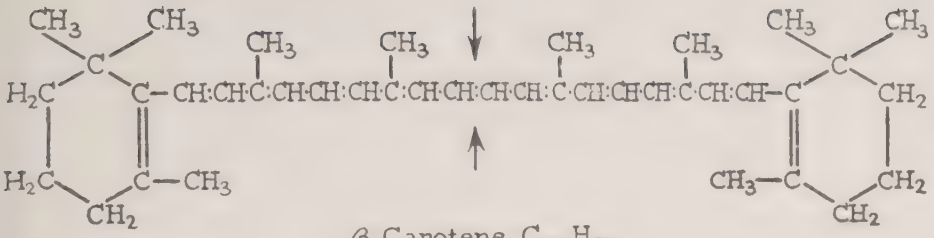
Small amounts of xanthophyll accompany carotene in milk, but the latter predominates. Because of the wide variation in carotene content of the ration of the dairy cow, there are extensive fluctuations in the carotene of milk. On a normal winter feed, milk fat contains about 0·3 mg. (milligrams) per cent. If, however, dried grass or good quality molassed grass silage are included in the ration, this value may rise two or threefold. It is significant that the quantity in the fat of colostrum may be ten or twenty times the normal, but apart from this the stage of lactation appears to have little effect.

The importance of carotene in milk fat is not only its colouring properties or its possible role as an anti-oxidant, but is due to the fact that it is known to be the precursor of vitamin A. During the course of animal metabolism carotene is broken at the central link of the long hydrocarbon chain, each half uniting with a molecule of water forming two molecules of vitamin A. Any excess of vitamin A is stored in the liver. It has been necessary to refer on other occasions to the profound physiological differences produced by slight modifications of chemical structure. This is again manifest in the carotinoids. While beta carotene gives two molecules of vitamin A, only one is produced from alpha carotene ; whereas the closely allied pigment xanthophyll gives none. Since the retina of the eye contains a carotinoid protein it may be expected that a lack of carotene or vitamin A in animal or human nutrition will have a serious effect on the eyesight of the subject.

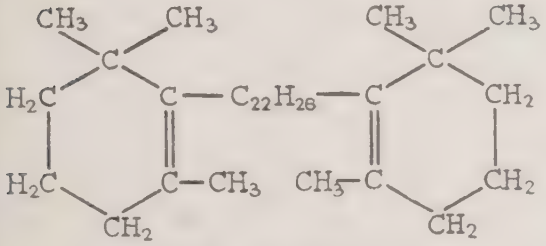
Because of the great importance of vitamin A in human nutrition the carotene of milk, cream, and butter brings these foods into the foremost rank. For the same reason great concern is occasioned by the low level of carotene in winter-produced milk, and determined efforts have been made in this country to correct this tendency. The more liberal winter feeding of kale, dried grass or good quality grass silage have yielded promising results. Reference will be made to this subject again in consideration of vitamins (page 46).

Gillam *et al.*[1] studied the seasonal variation in carotene and vitamin A and obtained results shown in the table. The figures illustrate the outstanding qualities of the Guernsey breed in giving

[1] Gillam, Heilbron, Ferguson, and Watson, 1936, *Biochem. J.*, 30, 1728.

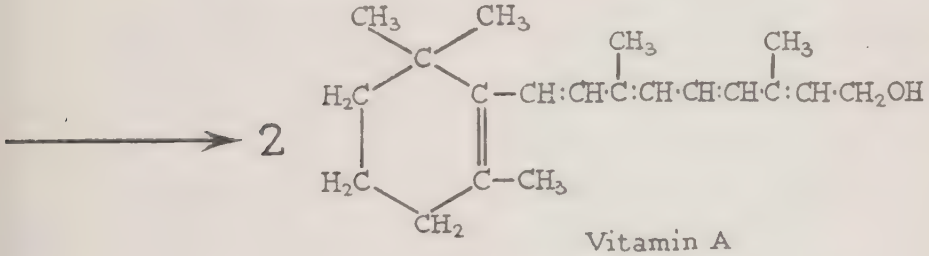


β Carotene $C_{40}H_{56}$

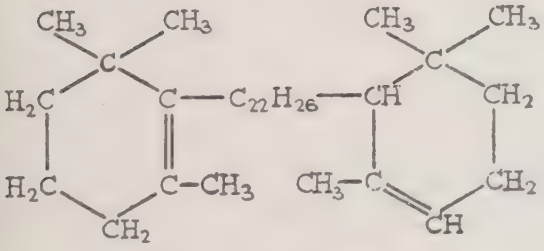


$+ 2H_2O$

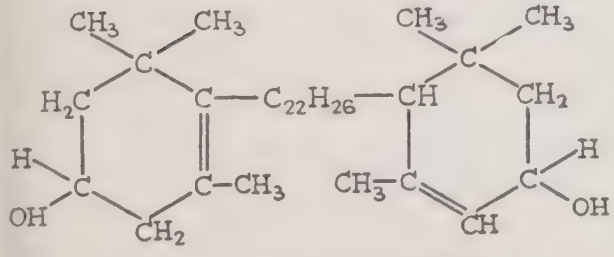
β Carotene



Vitamin A



α Carotene $C_{40}H_{56}$



Xanthophyll $C_{40}H_{54}(OH)_2$

milk fat of much higher carotene content, although comparison between the breeds is difficult on account of the wide difference between individuals. Thus the figures of Sharp and Hand[1] while confirming these general observations on the effect of breed and season, also demonstrate the wide range of individual variation.

CAROTENE AND VITAMIN A CONTENT OF MILK FAT AS INFLUENCED BY BREED AND FEEDING

(Gillam *et al.* 1936.)

mg. PER 100 GMS. OF FAT

	Gross average	Winter	Summer	Ratio Vit. A/ Carotene
Shorthorn:				
Carotene	0·27	0·25	0·29	2·5
Vitamin A	0·68	0·58	0·84	
Ayrshire:				
Carotene	0·36	0·27	0·41	2·4
Vitamin A	0·85	0·66	1·18	
Friesian:				
Carotene	0·40	0·35	0·47	2·25
Vitamin A	0·90	0·61	1·21	
Guernsey:				
Carotene	0·92	0·73	1·14	0·80
Vitamin A	0·75	0·54	0·95	

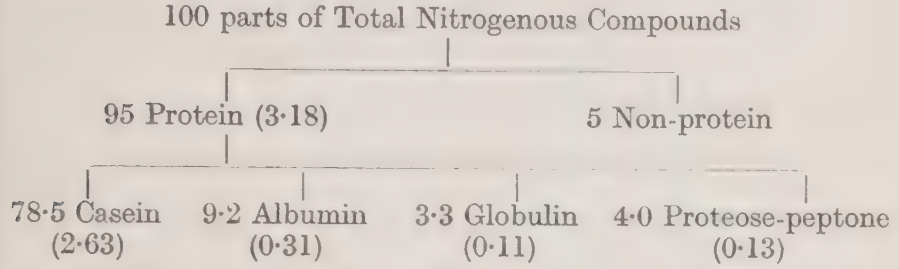
DATA OF SHARP AND HAND 1939

mg. CAROTENOIDS PER LITRE OF FAT

	Winter			Summer		
	Samples	Range	Average	Samples	Range	Average
Guernsey	13	4·68–12·0	8·5	13	10·4 –20·8	16·4
Jersey .	25	4·15–11·5	5·87	21	6·55–14·2	10·5
Ayrshire	11	1·93– 9·59	4·27	8	5·77– 9·85	6·58
Holstein	20	1·45– 9·42	3·49	17	2·72–10·6	5·78
Herd .	72	1·45–12·0	5·42	62	2·72–20·8	9·95

[2] Sharp and Hand, 1939, *J. Dairy Sci.*, 22, 737.

The Nitrogenous Compounds of Milk.—Normal milk contains approximately 0·5 per cent of nitrogen which is distributed within a number of compounds as shown in the table.



The figures in brackets represent average percentages in milk as determined by Rowland.[1] The distribution of nitrogen is considerably altered by physiological abnormalities and indeed is utilised in the chemical diagnosis of mastitis milk.

Milk Proteins.—Before commencing a detailed study of the milk proteins a general survey of the important group to which they belong will help to place them in their true perspective.

Proteins are without exaggeration the most complex substances known to chemical science, and it is therefore not surprising that even with the many improved methods of investigation which are at present available, their precise constitution still remains to be established. They contain the elements C, H, O, N, in the following approximate proportions: 52 per cent, 7 per cent, 22 per cent, 16 per cent, respectively. Sulphur may be present up to about 2 per cent and phosphorus is in certain members in amounts approximating to 1 per cent.

It is only possible to make an intelligent guess at their molecular weights and formulæ. For instance, Svedberg's ultra-centrifugal studies led to the conclusion that most native proteins possess molecular weights bearing a simple ratio to 34500 which represents the albumin molecule. Given that any particular protein contains the elements C, H, O, N, S in accurately determined proportions, an attempt may be made to calculate its empirical formula on the assumption that at least one atom of sulphur will be included in the molecule. Such attempts result in formulæ of enormous dimensions in which any one molecule may be composed of several thousand atoms. Until more is known of the internal design of the protein molecules such formulæ can possess little real significance. Actually the importance of proteins in

[1] Rowland, 1938, *J. Dairy Res.*, 9, 47.

the natural world depends almost entirely on the simpler amino acids of which they are composed. In animal and plant metabolism, in the ripening of cheese, in the process of digestion or bacterial proteolysis, it is these amino acids which are of utmost importance.

On hydrolysis, proteins furnish a mixture of amino acids, of which glycine (amino acetic acid) may be taken as the simplest example. Its formula is CH_2NH_2COOH and the general formula $R.NH_2COOH$ may be taken to represent the group provided it is realised that variations in the numbers of amino (NH_2) and acid (COOH) groups occur. These amino acids are able to unite with the elimination of water much in the same way as simple carbohydrates combine to form complex starches, e.g.

$$nC_6H_{12}O_6 - nH_2O = (C_6H_{10}O_5)_n$$

Two molecules of the same or different amino acids unite by eliminating water from the COOH group of one and the NH_2 group of the other, e.g.

$$NH_2 - CH_2 - CO|OH| \quad H|NH - CH_2 - COOH = NH_2CH_2CONHCH_2COOH + H_2O$$

Glycine — Glycyl glycine

The resulting di-peptide still contains the antagonistic groups (NH_2) and (COOH), so that the process of building up more complex molecules may proceed in the same manner. There is little doubt that this is one of the stages in the formation of the protein molecule. On account of the fact that the NH_2 group of amino acids possesses basic properties whereas the COOH is acid by nature, these compounds are able to combine with both acids and bases. This amphoteric reaction is shared by the proteins by virtue of the fact that however complex they may be, some of these reactive groups are sufficiently exposed to allow the protein to manifest amino acid properties.

While it would be out of place in a book of this description to embark on a discussion of the manifold properties of proteins, mention must be made of those which play a dominant part in milk and dairy products.

Because of their enormous molecular dimensions proteins behave as colloids and show the phenomenon of adsorption. They are also able to imbibe considerable quantities of water so that although solid in the pure condition, in solution the particles are

in the fluid condition. Thus the ultimate particles of colloidal protein solutions are really very concentrated protein/water mixtures possessing gelatinous or fluid properties. Such colloidal solutions are termed emulsoids. It follows that in most natural fluids containing proteins some of the water is bound. For example, some part of the 87 per cent of water in milk is not in the free condition, being bound tenaciously by the milk proteins. The significance of bound water in dairy products will be appreciated in a later chapter.

In their ability to unite with metals, proteins show a characteristic of great importance. The precise mode of combination is still obscure. While the simplest view is that the metal is united in the normal way to one or more of the COOH groups, it is by no means certain that this is the case, for these groups are woven into a colloid particle of great complexity. Adsorption will undoubtedly fix some of the metal to the protein surface. Whatever may be the correct explanation it is necessary to appreciate that the solubility of most metals in water is considerably increased by the presence of protein owing to the fact that in the metal proteinate, the metal is virtually thrown out of solution. Thus the equilibrium [metal $\rightleftharpoons$ metal in solution] is disturbed and more metal goes into solution in an endeavour to restore the balance. This mechanism assumes practical importance in the study of corrosion of metals by dairy products and the consequent spoilage of plant and product.

The amphoteric nature of the proteins allows them to function as mild buffer compounds: i.e. they are able to resist to some extent the change of hydrogen ion concentration (pH) which would normally result from the addition of acids or bases to equivalent quantities of water.

Although proteins in solution are essentially colloidal, the outer exposed groups do manifest a very slight degree of ionisation. For each protein there exists a certain pH at which this ionisation is at a minimum and at which the protein is least soluble. Therefore its separation from solutions is most complete at this particular pH which is known as the iso-electric point.

One of the nutritive functions of proteins is to furnish a number of amino acids which may be transformed by the animal into body or milk proteins. Further as they differ in the number and nature of their constituent amino acids, it follows that according to the precise needs of the animal for particular amino acids, the

proteins from different sources may possess different nutritive values. Thus in human and animal nutrition, one is often brought face to face with the necessity for discrimination between *quantity* and *quality* of food protein. Many experiments have proved that animals may consume a ration containing the correct quantity of protein, yet because one or more essential amino acid is lacking, growth ceases and symptoms of starvation are in evidence. It will be seen in the consideration of milk proteins that their high nutritive value lies in the fact that they supply essential amino acids and are thus able to correct deficiencies in other common human and animal foods.

The Milk Proteins.

Casein.—This compound may be defined as the protein which is precipitated from milk at a *p*H of 4·6. Some confusion may arise over two systems of nomenclature, for while British workers have used caseinogen to denote milk protein as distinct from casein the cheese curd protein, the American usage is to denote the former protein by the name casein and the latter by para casein. For some time there has been a general drift towards the American system, which it is proposed to follow in this text-book. The elementary composition of this and the other two milk proteins as determined by Van Slyke and Bosworth[1] is given in the table, from which it will be seen that casein belongs to the phosphoproteins.

Composition of the Milk Proteins

	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus
Casein .	53·5	7·13	22·14	15·8	0·72	0·71
Albumin	52·51	7·10	23·04	15·43	1·92	——
Globulin	51·88	6·96	24·62	15·44	0·86	0·24

Early investigations into the composition of casein were hampered by the difficulty experienced in separating it from calcium phosphate with which it is so intimately associated in milk. Thus, the quoted figures for phosphorus content show some variation on that account. The inclusion of the phosphoric acid radical in the molecule gives casein valuable nutritive properties and is believed to be the cause of its exceptional acid qualities. Although it shares with other proteins the ability to

[1] Van Slyke and Bosworth, 1913, *J. Biol. Chem.*, 14, 203.

form salts with both acids and bases, its acid nature predominates in milk. It combines with sodium hydroxide to form sodium caseinate, and is sufficiently acid to liberate carbon dioxide from calcium carbonate. The base-binding powers have been estimated as approximately 9 ml. N/10 NaOH per gm. casein. It has been suggested that some at least of this acidity may be attributed to residual acid groups of the phosphoric acid molecule.[1] In cow's milk casein is united to calcium, but the acid properties are only partially nullified thereby, the resulting compound being an acid calcium caseinate or more correctly, calcium hydrogen caseinate. Although there may be certain theoretical objections to this picture of the casein molecule as it exists in milk, it does help the student to understand the chemical reactions of casein in milk and dairy products.

Like all acid salts, the residual acid may be neutralised by the addition of a base. The addition of sodium hydroxide to milk will therefore neutralise calcium hydrogen caseinate in the same way as its addition to a solution of acid sodium phosphate will result in the formation of the base saturated tri-sodium phosphate.

$$\text{Calcium acid caseinate} + \text{NaOH} = \text{Calcium} - \text{sodium} - \text{caseinate} + \text{Water}$$

It must follow therefore, that when titrating milk with sodium hydroxide as in the customary acidity test, some of the solution will be used to neutralise casein, quite irrespective of the presence or absence of lactic acid. The author has found that the acidity of casein as it exists in milk amounts to approximately 0·32 ml. of N NaOH per gm. of casein.[2] On this reckoning about one-third of the acid groups of casein are normally free in milk.

It is well known that as lactic acid develops in milk, there is a gradual migration of calcium from the colloidal to the soluble condition, and that this process continues until the sample curdles. There are two reactions which account for this enrichment of the soluble phase at the expense of the colloidal, one which is the removal of calcium from calcium hydrogen caseinate to form calcium lactate according to the equation :

$$\underbrace{\text{Calcium hydrogen caseinate}}_{\text{Colloidal}} + \text{Lactic acid} = \underbrace{\text{Acid casein}}_{\text{Precipitate}} + \underbrace{\text{Calcium lactate}}_{\text{Soluble}}$$

[1] R. A. Gortner, 1938, *Outlines of Biochemistry*, p. 481.
[2] E. R. Ling, 1937, *J. Dairy Res.*, 8, 173.

This equation represents the state of affairs at the iso electric point, i.e. at *p*H 4·6. Before this degree of acidity is reached there is a progressive removal of calcium from the protein, although the latter is not precipitated until the correct *p*H is attained.[1] The precipitated protein includes fat and a certain amount of the whey. *Although it is possible to make cheese in this manner, the reaction is not the same as occurs when milk is coagulated by rennet, which converts the casein into a new protein. The physical and chemical nature of these two curds is very different.* Rennet coagulation will be studied under the heading 'Cheese'. It is not necessary to allow milk to sour naturally in order to precipitate pure casein; indeed, such a procedure would be open to the objection that a certain amount of proteolysis may occur, according to the nature of the micro-organisms present. The analytical method for estimating casein depends upon the addition of dilute acetic acid and sodium acetate as a buffer to bring the *p*H to 4·6, whereupon a clean and quantitative separation of pure casein occurs.

The other reaction which is involved in the conversion by lactic acid of colloidal calcium to the soluble form is concerned with tri-calcium phosphate, which is bound to casein by physical forces. It is necessary to complete the picture of casein as it exists in milk by visualising colloidal tri-calcium phosphate held by surface forces to the colloidal calcium hydrogen caseinate; for the associated phosphate enters into all reactions involving casein. It will be seen when dealing with the action of rennet that the tri-calcium phosphate plays an important part in determining the physical nature of rennet curd. The nature and mode of attachment of the associated phosphate have been the subject of many researches. Although at one time believed to be di-calcium phosphate, $Ca_2(HPO_4)_2$, there is evidence to justify the view that it is the basic tri-calcium phosphate which is held by casein.[1] [2] [3] Thus, when neutral potassium oxalate is added to milk there is a reduction in acidity due, in part, to the reaction:

$$\underset{\text{Colloidal}}{Ca_3P_2O_8} + \underset{\text{Neutral}}{3K_2 \text{ oxalate}} \rightleftharpoons \underset{\text{Precipitate}}{3Ca \text{ oxalate}} + \underset{\text{Alkaline reaction}}{2K_3PO_4}$$

The percentage of colloidal $Ca_3P_2O_8$ in milk is estimated to be approximately 0·15.[1] [2] [3]

As lactic acid develops in milk, there is a progressive removal

[1] E. R. Ling, 1936, *J. Dairy Res.*, 7, 145. [2] Pyne, 1934, *Biochem. J.*, 28, 940.
[3] Ling, 1937. *J. Dairy Res.*, 8, 173.

of the phosphate from the surface of casein, according to the equation:

$$\underset{\text{Colloidal}}{Ca_3P_2O_8} + 4CH_3CHOHCOOH = \underset{\text{Soluble}}{CaH_4P_2O_8} + 2\underset{\text{Soluble}}{(CH_3CHOHCOO)_2Ca}$$

Again the soluble phase is enriched at the expense of the colloidal, on this occasion mono calcium phosphate entering the soluble state along with calcium lactate.

Although this section is not devoted to lactic acid development it may be advantageous to summarise the main effects as follows:

1. Decrease of lactose concentration.
2. Progressive conversion of colloidal tri-calcium phosphate attached to casein into soluble mono calcium phosphate and calcium lactate.
3. Gradual removal of calcium from calcium hydrogen caseinate to form calcium lactate and a more acid casein.
4. As a direct result of the foregoing effects, an increase of osmotic pressure and consequent decrease of freezing-point.
5. An increase in hydrogen ion concentration (i.e. the *p*H figures show a decrease).
6. At *p*H 4·6 a precipitation of Casein.

For some years there has been an accumulation of experimental data which would indicate that casein is a mixture of at least three constituents showing differences in solubility, chemical composition and in their behaviour towards rennet. The experimental work is difficult and beset by many possible errors, and those interested in the details should consult the original papers.[1] Although the case cannot be considered proved beyond all doubt, there are several properties of milk which find best explanation on the hypothesis that the principal protein is a mixture. For instance as long ago as 1874, Hammarsten found that when casein is clotted with rennet, a small amount of soluble nitrogenous matter passes from casein into the whey. This small fraction, representing some 4 per cent of the casein, has stimulated keen interest and controversy, and is generally known as Hammarsten's proteose. Cherbuliez and Jeannerat resolved casein into three fractions, one of which, representing about 4 per cent of the original, is not coagulated by rennet and therefore escapes

[1] (McKee and Gould, 1938. *J. Agric. Res.*, 57. 125; Cherbuliez and Jeannerat, 1939. *Helv. chim. Acta*, 22, 952; Mellander, 1939. *Biochem. Z.*, 300, 240.)

into the whey, while the other two are coagulated. The significance of this finding will be better appreciated when considering the action of rennet on milk, but it may be stated here that the conception of rennet attacking casein by releasing the soluble fraction and precipitating the remainder is one which opens up an entirely new field of study.

The solubility of casein in various solvents is also a matter of industrial importance in the preparation of casein glues and cements. As early as the eleventh century a water-resistant glue was made from mixtures of curd and hydrated lime. In borax solution casein dissolves to form a glue which possesses many advantages over bone glue. Sodium hydroxide solution dissolves casein to give sodium caseinate, which may be forced through fine jets into a precipitating medium whereby casein fibres are obtained. Casein textiles known as Lanital are comparatively new and their future development will be followed with keenest interest.

Great interest is also shown in the development of casein plastics, which have found their way into thirty or forty trades, and from which such familiar articles as buttons, insulating panels, fountain-pen barrels, umbrella handles, piano keys, knife handles, combs, knitting needles, etc., are frequently made. It must be emphasised, however, that casein is only one member of a growing list of substances named plastics. In the late nineteenth century it was discovered that casein reacts with formaldehyde to produce a new compound possessing properties very distinct from those of the parent material. It is considered that the reaction concerns the free NH_2 groups of the protein, although the precise mechanism of the reaction is still obscure. The treatment renders casein much more resistant to water, acids and heat.

Although the raw material for casein plastics is rennet casein, it is possible to prepare them from acid precipitated casein, but owing to lower production costs, the former is generally used. The dried material is ground to the requisite fineness, coloured, mixed with the minimum amount of water and subjected to heat and pressure. As a result of this treatment the substance softens and may be extruded from machines in the form of rodding or tubing. Sheeting may be made by hydraulic pressure on the heated rods. The material is then hardened by immersion in 4–5 per cent formaldehyde for varying lengths of time,

sometimes two or even ten weeks being required. The hardened product, although insoluble in water, is somewhat hygroscopic and therefore is not used for high-tension insulation or outdoor work. It is not inflammable and may be turned, drilled, polished, stamped, engraved, or moulded. If the coming decade is to see plastics come into their own it will be interesting to note how far casein is able to compete with other synthetic types. As the raw material for commercial casein is separated milk, it is obvious that the supply is strictly limited.

Mention should be made also of the use of casein in cold-water paints, horticultural sprays and for putting a finish on paper and leather.

When casein is mixed with hydrated lime and is ground with water in a mortar, a milky solution of calcium caseinate is obtained. From this solution casein 'milk' may be obtained by gradual addition of phosphoric acid until a *p*H of 6·3 is reached. That this colloidal solution is coagulated by rennet and forms a skin on heating certainly points to the calcium caseinate-calcium phosphate complex as the origin of such changes in milk. Both will be discussed later.

It is sometimes stated that casein differs from albumin and globulin in so far as the former is not coagulated by heat. This statement is not correct, for one of the problems of the evaporated milk industry is the prevention of heat coagulation during the final sterilisation of the product. In its fresh condition milk autoclaved at 270–280°F. will show heat coagulation of its casein. There are many factors operating in this change, amongst which are acidity, and mineral balance. They will be discussed more fully in a later chapter, but it is desired to emphasise here that casein *can* be coagulated by heat.

The high nutritive value of casein is widely recognised. It is a complete protein supplying all the essential amino acids for human and animal needs, and is a valuable complement to the cereal proteins which form a large part of human dietaries. It is used in a number of proprietary foods, invalid diets, etc., while in medicine it is a useful conveyor of iodine or heavy metals owing to its ready combination with these elements.

Lactalbumin and Globulin.—These two proteins, constituting about one-eighth of the total, are usually described as the soluble proteins of milk. This is of course a very superficial distinction, for all the milk proteins are in the colloidal form; but it does

serve to emphasise that whereas dilute acids or rennet will remove casein from solution, the remaining proteins are unaffected. Although in milk both are dissolved, they differ in that while albumin is soluble in distilled water, globulin is insoluble in water but soluble in dilute salt solutions. Both are coagulated by heat, but the extent of such coagulation is governed by temperature, time of holding, salt concentration, and *p*H of the solution. At 65°C. heat coagulation commences and increases in extent as the temperature and time of holding are increased, a *p*H of 4·5 representing the most favourable reaction for the change. Orla Jensen[1] considers that when the temperature exceeds 90°C., some of the heat coagulated protein re-dissolves and in so doing gives the typical cooked flavour of heated milk.

The chemical constitution of these two proteins has been the subject of much enquiry, particularly with a view to establishing whether or not they are identical. with the corresponding albumin and globulin of blood and colostrum. Although the globulin of milk and blood are identical, the respective albumins though similar, are not identical. Thus it would appear that the mammary gland allows a very small amount of globulin to filter into the milk from the blood stream, but is obliged to elaborate lactalbumin from the blood constituents. So far as colostrum is concerned both albumin and globulin are the same as those present in milk, but there is a much larger quantity of globulin. Whereas in milk the concentration of globulin is as low as 0·1 per cent, in colostrum it may be as high as 6 per cent—high enough to cause visible coagulation on boiling. This property is lost as the globulin gradually declines to the normal level.

Reference to the table on page 34 shows that phosphorus appears in the elementary analysis of globulin. This has been and is still a matter for further elucidation, and while it is not possible to state its precise significance, it is probable that the phosphorus belongs to a phospholipid which is incorporated in the globulin fraction.

These two proteins possess a high nutritive value, supplying the essential amino acids. Moreover, albumin is able to rectify to some extent the rather low sulphur content of casein. Thus it is that the nutritive value of the three milk proteins in the presence of one another is higher than when measured separately. They are in fact complementary.

[1] Orla Jensen, 1928, *Proc. World's Dairy Congress.*

Other Proteins in Milk.—The existence of traces of other proteins in milk is generally accepted. In the study of the fat globule surface, the presence of a riboflavin-protein-phosphoric acid complex was established.[1] Sharp and Hand[2] estimate that 0·07 gm. of this material is absorbed by 100 gms. of fat (i.e. approximately 0·0025 per cent in milk).

It is quite possible that there are more than one flavo protein in milk. They are reddish brown in colour, but colourless in the reduced condition. When cream is churned they pass into the buttermilk. Their great reactivity in oxidation—reduction changes makes them the focus of much attention, and it is interesting to find that they may be absorbed by the fat globules where it is likely that they take part in the chain reactions associated with fat oxidation.

Other substances of a protein nature are the milk enzymes. Although their total weight percentage is undoubtedly very small this must not detract from their importance; for being catalysts, they are able to bring about important reactions in very low concentrations.

After the removal of casein, albumin and globulin from milk, the filtrate still contains a nitrogenous compound which may be precipitated by saturation with ammonium sulphate. Rowland considers that these proteose-peptone substances are normal milk constituents. Since proteoses, peptones, and amino acids are produced by the degradation of proteins there has always existed some doubt as to whether this proteose-peptone is a normal milk constituent, or whether it has appeared as a result of the analytical separation of the proteins. However, it is now generally agreed that it is a normal constituent. Whether it is in any way related to the proteose fraction of casein cannot yet be stated.

The behaviour of the three proteins towards some of the common laboratory reagents is shown in the table on p. 42.

Non-Protein Nitrogenous Substances.—These, amounting to about 5 per cent of total nitrogen, include a variety of substances such as amino acids, certain vitamins, urea, uric acid, ammonia, creatine, creatinine, etc. It will be appreciated that the total concentration being equivalent to about 0·025 per cent nitrogen, the quantities of each member must be very small indeed. These, together with globulin, filter into milk from the blood and under

[1] Corran, 1938, *Biochem J.*, 32, 2231.
[2] Sharp and Hand, 1939–1940, *Rep. Cornell Agric. Exp. Sta.*, p. 121.

	Casein	Albumin	Globulin
Rennet	Coagulated	Not coagulated	Not coagulated
Heat	Coagulated above 100°C	Coagulated	below 100°C.
Cold dilute acids at *p*H 4·6	Precipitated	Not precipitated	
Excess ammonium sulphate . . .	Precipitated	Precipitated	Precipitated
Equal volume of saturated ammonium sulphate	Precipitated	Not precipitated	Precipitated
Excess sodium chloride .	Precipitated	Not precipitated	Not precipitated
Excess magnesium sulphate . . .	Precipitated	Not precipitated (unless acid)	Precipitated

normal circumstances their concentration is low. Physiological disturbances such as mastitis result in a much higher permeability of the membranes, and consequently the level of soluble nitrogen increases considerably.[1] The casein number $\left[\frac{\text{Casein N}}{\text{Total N}} \times 100\right]$ then falls below the normal value of 79. A fuller consideration will be given to this subject on page 92.

The Mineral Matter of Milk.—Emphasis has already been laid on the fact that in considering the constituents of milk one is examining individual building stones, and that it is often impossible to do more than imagine how they are arranged in the design of the complete edifice. This is very true of the mineral matter.

By evaporation of milk to dryness followed by gentle ignition a grey ash is obtained which, on analysis, reveals the presence of phosphates, chlorides, potassium, calcium, and sodium in appreciable quantity, with smaller amounts of magnesium, sulphate, carbonate, and a trace of iron. Very minute traces of copper, aluminium, boron, zinc, manganese, and silicon have been reported by various workers. For the moment it is necessary to enquire into the precise relationship between the ash and the milk from which it is derived.

[1] W. L. Davies, 1932, *J. Dairy Res.*, 4, 142.

The ash is distinctly basic, the total equivalents of the basic constituents being in excess of the acids, and as milk is normally only slightly acid it is clear that the very process of ashing has altered the original mineral balance. These alterations are as follows:

1. Phosphates of the ash have been augmented by some P_2O_5 derived from organic phosphorus.
2. Sulphates of the ash have been derived from the burning of proteins.
3. Similarly carbonates are present by the union of CO_2 with the bases.
4. Ignition of citrates normally present in milk results in their conversion to carbonates.
5. Unless the temperature of ignition is kept below dull red heat, some loss of alkali chlorides occurs by volatilisation.
6. The tracesof iodine normally present in milk are lost in ashing.
7. While normal milk gives about 0·76 per cent ash, it is certain that the total of mineral salts in milk will exceed this figure.

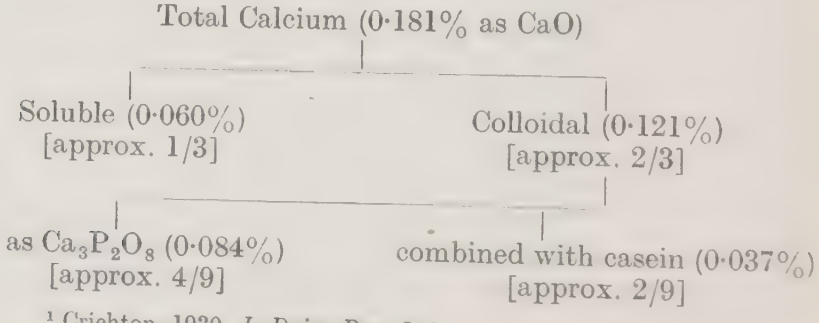
Very few complete mineral analyses have been made, so that a composite of many different investigations must serve to present the complete mineral picture. The figures given in the table on p. 44 have been compiled from a study of data at present available. The figures in the second column give the maximum, minimum, and average percentages of the major mineral constituents of 220 samples of milk from a herd of 20 Ayrshire cows over three lactation periods. They represent the most complete mineral survey at present available. It must be emphasised that the figures are very variable, being influenced particularly by lactation, season, physiological disturbances, and in some instances by feeding. These will receive treatment elsewhere (Chapter III).

Many attempts have been made to construct the complete salt balance of milk from the quantities of the various radicals, but when one considers that some of the constituent salts exist in the ionic condition, some as unionised molecules some in the colloidal state, and some in more than one of these physical conditions, the difficulty of the problem is at once apparent. Indeed, some authors contend that such attempts are futile. Although it may not be possible to construct the complete salt balance with any degree of confidence, a more practical approach to the problem may be made by a consideration of the distribution

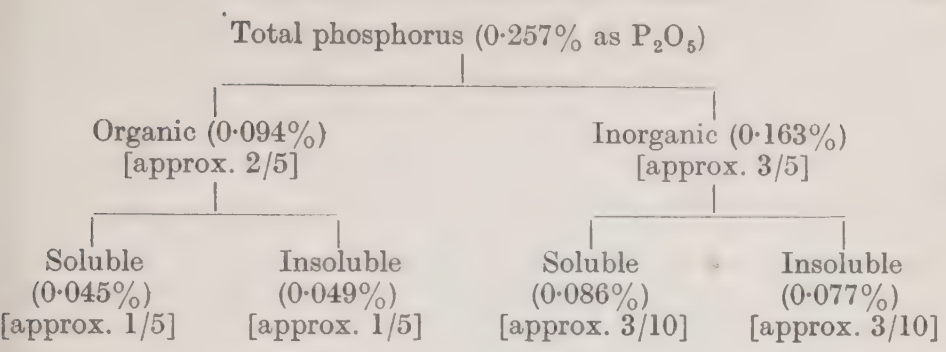
Mineral constituents as compiled from many different sources		Analysis of 220 samples of milk from 20 Ayrshire cows.[1]		
	Estimated average as % milk	Average	Minimum	Maximum
K_2O . . .	0·190	0·202	0·148	0·217
CaO . . .	0·176	0·166	0·126	0·212
Na_2O . . .	0·068	0·076	0·050	0·155
MgO . . .	0·018	——	——	——
P_2O_5 . . .	0·233	0·229	0·166	0·291
Cl . . .	0·106	0·111	0·068	0·163
Citrate expressed as citric acid	0·18	——	——	——
Fe . . .	1–2 parts per million	——	——	——
Cu . . .	0·12 parts per million	——	——	——
I . . .	0·02–0·14 parts per million	——	——	——

of the major constituents between the soluble and colloidal phases.

The chlorides of potassium and sodium are in true solution, whereas the phosphates of calcium and magnesium are partly colloidal and partly soluble. In our study of casein it was apparent that a considerable amount of the total calcium and phosphate was associated with this protein and therefore appeared in the colloidal phase. Van Slyke and Bosworth[2] found that the ratio of soluble/total calcium and phosphorus in milk varied with the individual and was to some extent influenced by the stage of lactation. The author[3] has determined the calcium and phosphorus distribution, which may be summarised as follows:


Total Calcium (0·181% as CaO)
Soluble (0·060%) [approx. 1/3]
Colloidal (0·121%) [approx. 2/3]
as $Ca_3P_2O_8$ (0·084%) [approx. 4/9]
combined with casein (0·037%) [approx. 2/9]

[1] Crichton, 1930, *J. Dairy Res.*, 2, 1.
[2] Van Slyke and Bosworth, 1916, *J. Biol. Chem.*, 24, 199.
[3] Ling, 1937, *J. Dairy Res.*, 8, 173.


Total phosphorus (0·257% as P_2O_5)
Organic (0·094%) [approx. 2/5]
Inorganic (0·163%) [approx. 3/5]
Soluble (0·045%) [approx. 1/5]
Insoluble (0·049%) [approx. 1/5]
Soluble (0·086%) [approx. 3/10]
Insoluble (0·077%) [approx. 3/10]

The proportion of these elements in the dialysable condition has been estimated by several workers, some of whom are quoted below.

	Ratio Dialysable/Total		
	Calcium	Phosphorus	Magnesium
	Per cent	Per cent	Per cent
Mattick[1] . .	24–30	29–39	—
Wardlaw[2] . .	30–40	35–55	—
Lampitt *et al.*[3] .	25–42	40–58	62–83

The presence of soluble organic phosphorus is accounted for by some of the phospholipid fraction and by other phosphoric esters at present unidentified. Some of the water-soluble vitamins also contribute soluble organic phosphorus, but the amount is extremely small.

Milk owes much of its nutritive properties to the mineral constituents. It is interesting to note that milk is actually richer in calcium than is lime water; in fact it may be considered as carrying a supercharge of this element. Milk thus becomes a good corrective to calcium-poor cereal diets of both man and animals, and is one of the most important sources of calcium in human nutrition. For children particularly, the value of milk as a source of the bone-forming elements, calcium and phosphorus, is at once apparent. All the mineral elements of milk are essential for nutrition, but it becomes necessary to emphasise that some, such as iron and iodine are not present in quantities which would justify the extravagant claim that milk is a perfect food. Regarded

[1] Mattick, 1929, *J. Agric. Sci.*, 19, 452.
[2] Wardlaw, 1914, *J. Roy. Soc. N.S.W.*, 48, 253.
[3] Lampitt *et al.*, 1937, *Biochem. J.*, 31, 1861.

as an excellent source of calcium and phosphorus, milk certainly occupies a prominent position amongst human foods, but claims to the attainment of perfection are absurd.

The mineral balance of milk is known to be one factor influencing the heat coagulation of casein. This is a matter of great importance in the final sterilisation of evaporated milk, where any abnormality in the balance Ca+Mg/phosphoric+citric acids is known to render the milk unstable at the sterilisation temperature. Rennet coagulation of milk is also partly dependent on the mineral distribution, but in this case it is the balance between soluble and colloidal calcium which operates. Both subjects will receive fuller consideration under the appropriate headings.

Considered as percentages of milk, the figures 0·18 and 0·23 for calcium and phosphoric acid respectively are not large, but when coupled with the yield per day, or the yield per complete lactation, they show that there is a very considerable loss of these elements by the dairy cow. Every 10 gallons of milk contain approximately 3 ounces of CaO and 4 ounces P_2O_5. This outgo being over and above that normally excreted, it follows that in compounding rations for heavy yielding cows, careful attention must be given to the supply of these two elements in an assimilable form. It cannot be assumed that a ration which supplies the correct amount of starch equivalent and protein equivalent will *automatically* give the required quantities of mineral matter.

The Vitamins of Milk.—The rapid growth of our knowledge of vitamins which has taken place during the past decade is still in the process of vigorous expansion and, in a book of this type it is impossible to give anything but a meagre treatment of the subject. It is hoped that the student will refer to the standard works on the subject for fuller information. Of these, Dr. Leslie Harris' *Vitamins in Theory and Practice* (Cambridge University Press) is recommended for those who require a general survey of the subject.

For very many centuries certain diseases such as scurvy, beri-beri and rickets have been known to attack the human race in various parts of the world, and although history records that occasionally methods of treatment had been devised, the origin of these human scourges remained obscure until comparatively recent times. Disease was considered to be the result of the *presence* of something malignant—be it an evil spirit, a germ, or a poisonous food or drink. The idea of a disease being caused by

the *absence* of something was one which the human mind could not readily conceive. Nevertheless the view that faulty feeding could have such disastrous results was slowly evolved and culminated in the pioneer work of men like Lunin, Funk, Stepp, Pekelharing, Hopkins, Osborne, Mendel, McCollum, and Davis.

In this country the experiments of Hopkins conducted in 1906 demonstrated the function of vitamins in nutrition. Selecting a batch of young male rats he fed them on a carefully purified diet of lard, casein, starch, sugar, mineral matter and water. These items were correctly balanced, and according to the accepted standards of the day the ration could be considered adequate. The rats failed to make satisfactory growth, and soon began to lose weight and condition. At this stage the inclusion in the ration of 2 ml. of milk produced phenomenal results. The rats showed immediate recovery and growth proceeded at the normal rate. On the other hand, a continuance of the chemically pure diet resulted in the death of the animals. This and many other experiments by Hopkins and other workers brought the conviction that a complete diet must contain substances hitherto unrecognised, other than fats, carbohydrates, proteins, mineral matter and water; and that these vital factors were present in whole milk.

Since these pioneer experiments were performed, research in the subject has been directed to the problem of isolating these various factors from common foodstuffs, studying their physiological functions and chemical nature, and to the possibilities of their synthesis in the laboratory or on a commercial scale. When the chemical nature of these vital factors (now known as vitamins) was obscure they were named "fat-soluble A," "water-soluble B," etc., but now that their chemical constitution is known, it is hoped that they will be called by their chemical names, particularly as the alphabetical system is not universally the same. As these notes are being written in the transition period, it will be necessary to use both systems.

The vitamins present in milk are:

Fat-soluble A, D, E (Tocopherol).
Water-soluble B_1 (Aneurin or Thiamin).
B_2 (consisting of Riboflavin, Nicotinic acid, Pantothenic acid, Pyridoxine or Adermin),
C Ascorbic acid.

In considering the milk vitamins it becomes necessary to distinguish between those which are merely present and those

which are in sufficient quantity to make milk a valuable source of supply. Thus, although all the above-mentioned vitamins are present, milk is regarded as an important supply of vitamins A and the B_2 group—at least so far as human nutrition is concerned. The small amounts of the remainder are valuable when supplemented by other foods.

Vitamin A.—The recognition of the fact that the yellow pigment carotene was the precursor of vitamin A has stimulated great interest in the effect of the ration on the carotene content of milk. As shown on page 30, the seasonal variation is very pronounced, and there is no doubt that liberal supplies of good quality green food are required to maintain a steady and adequate level of carotene in milk. Watson and Ferguson[1] found that the feeding of dried grass or molassed silage was able to bring the carotene content of winter milk well up to the summer level. On a control ration of roots, hay, and concentrates the average carotene content amounted to 0·26 mg. per 100 gms. fat, whereas on the dried grass and molassed silage rations these values rose to 0·85 and 0·66 respectively. During the twelve weeks of the experiment, there was a steady decline in carotene content of the control milks, but with the two experimental rations the level was well maintained.

This vitamin is necessary to maintain the tone of the mucous membrane, which, in the absence of vitamin A, undergoes a process of 'hardening,' rendering the subject prone to infection of the nose, throat, lungs, and ears. In dealing with carotene it was seen that the retina contains a carotenoid protein, and it is not surprising that lack of vitamin A or carotene should result directly or indirectly in such visual disorders as night-blindness and Xerophthalmia. The view is sometimes expressed that vitamins are needed only by the young. While it is true that the need for vitamin A is greatest during growth, there is ample evidence to show that it is also required to maintain good health in the adult.

The vitamin is purely vegetable in its origin. Any excess above the normal requirements is stored in the liver, and as the animal cannot synthesise it, this mechanism affords some measure of protection against vitamin A starvation at such periods of the year when the supply of vegetable matter is low. Foods which are relied upon to supply vitamin A are liver oils, milk fat, egg

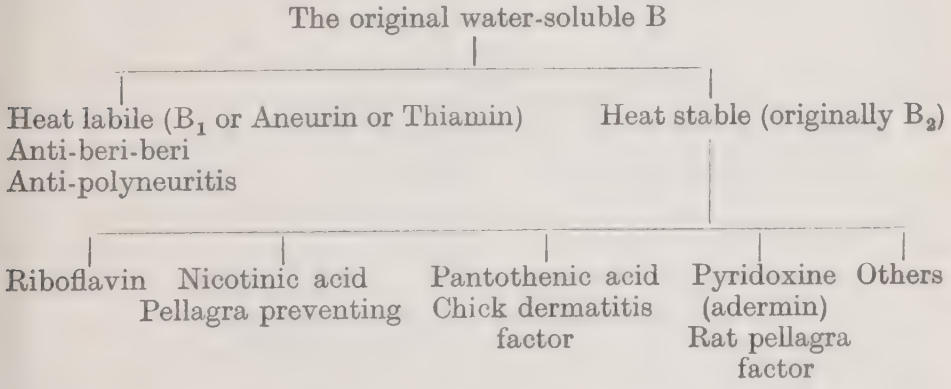
[1] Watson and Ferguson, 1936, *J. Agric. Sci.*, 26, 337.

yolk, green foods, and carrots. Most oils from oil seeds are deficient in this vitamin, a serious matter to the margarine manufacturer, who uses these oils extensively. Concentrated preparations of vitamin A are now available and these are incorporated in margarine to maintain the product at a steady all-the-year level; indeed, in the Second World War this was required by law.

Contrary to earlier views, vitamin A is fairly heat stable, neither pasteurisation nor sterilisation causing any appreciable reduction in quantity. Similarly, condensation and drying are without effect. Being highly unsaturated compounds, vitamin A and carotene are susceptible to oxidation. When butter fat undergoes oxidation the natural colour of the carotene is simultaneously bleached. For this and other reasons it is desirable in any form of milk processing to reduce risks of oxidation to a minimum.

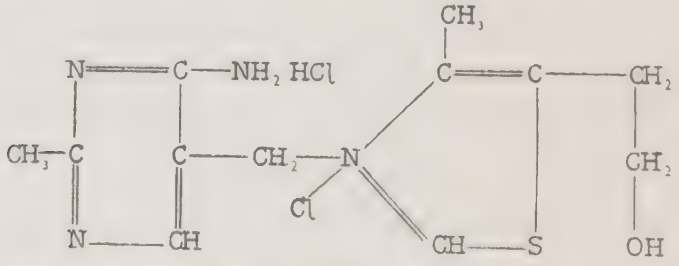
An interesting circumstance arises from the vitaminisation of margarine. Unless determined efforts are made to improve the winter feeding of the dairy cow, winter butter will compare unfavourably in vitamin A potency with vitaminised margarine. With the rapid disappearance of public prejudice against margarine there is likely to be a lively competition between the two industries unless steps are taken to improve the winter milk.

Vitamins of the B Group.—Water-soluble B was originally considered to be one substance, but it was later resolved into two sections, one heat labile (B_1), and the other heat stable (B_2). The process of fractionation continues, and we now recognise vitamin B_2 as a mixture of a number of vitamins. As the position is still rather confused, the student will find the following diagram useful.



Aneurin, Thiamin or Vitamin B_1.—(*Vitamin B in America.*)—This substance is the anti-beri-beri vitamin, the absence of which

brings about a form of paralysis of man (beri-beri) and animals (polyneuritis). It has other important functions, such as growth stimulation, maintaining the tone of the alimentary canal, and is known to be actively concerned with carbohydrate oxidation. Although the cow may obtain small quantities of thiamin by bacterial action in the rumen, most animals depend upon the food to supply this vitamin. In milk it exists partly in the diffusable condition either free or combined with phosphoric acid, and partly fixed as compounds of proteins with either of these forms. Colostrum contains 0·06–0·10 mg. per cent, early milk

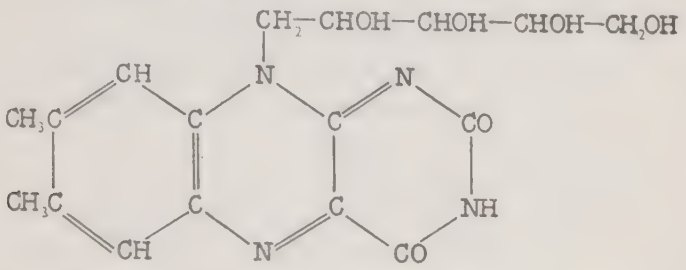


about 0·06 mg. per cent, and later milk between 0·03–0·04 mg. per cent. There is thus a rapid decline during early lactation. Season and feeding appear to be without effect.[1] Richer sources of this vitamin are the whole cereal grain, eggs and yeast. Being situate in the germ or adjacent layers, thiamin is to a large extent removed from the cereal grain during the process of milling in which a white flour is the aim. Thus it becomes necessary in time of war either to produce a white flour which must be enriched with vitamin preparations or to insist upon the milling of flour which contains sufficient "bran" to ensure a reasonable supply of thiamin.

Riboflavin.—(*Vitamin G in America*).—This compound is identified with the green yellow pigment of whey, and is also known to exist as a phosphoric acid-riboflavin-protein complex to which reference has already been made. It is considered that one-tenth of the 0·1 mg. per cent of riboflavin in milk is bound to protein.[1] There is a higher concentration in colostrum (0·4–0·4 mg. per cent), but the level declines very rapidly to the normal for milk. The quantity in milk appears to rise under summer feeding conditions. The possibility of this substance being synthesised like thiamin in the rumen must not be overlooked. Riboflavin is also present in liver, kidney, eggs, and green foods.

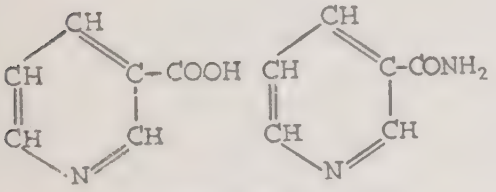
[1] Houston *et al.*, 1940, *J. Dairy Res.*, 11, 145.

Amongst its many functions, it is a growth-promoting health-stimulating substance which is strongly identified with oxidation-reduction changes where it plays the rôle of a catalyst or a co-catalyst. It is obviously a substance of vital importance in

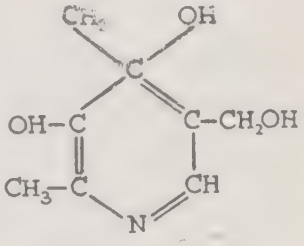


plant and animal metabolism. In the destruction of vitamin C in milk by oxidation under the influence of light, riboflavin is known to act as a sensitiser.

Nicotinic Acid and its Amide.—In 1937 this substance was shown to be the vitamin which prevents a disease known as pellagra. This disease in which serious skin disorders, gastro-intestinal symptoms, and dementia are manifest, attacks the poor sections of the community in the southern United States and in the East, although occasional outbreaks elsewhere have been reported. Phenomenal cures were effected through the oral administration of nicotinic acid or its amide. Liver, kidney, milk, eggs, and whey are good sources of this vitamin. It is significant that nicotinic acid amide is associated with riboflavin in a number of oxidation-reduction enzymes.



Nicotinic Acid and its Amide



Pyridoxine

Pyridoxine (Adermin).—This substance functions as the rat pellagra preventive. It is a member of the B_2 group and is present in milk, but it is not as yet known whether it possesses other important properties.

Pantothenic Acid.—This member of the group has been identified as the anti-chick dermatitis factor. At present it is being

used for the cure or prevention of white or grey hair. This substance is an important growth factor for yeast.

Having such important functions to fulfil in plant and animal nutrition, it is not surprising that the B group should be necessary growth factors for micro-organisms. Members of the B_2 group are much more heat stable than is thiamin. Whereas pasteurisation and sterilisation of milk cause losses of thiamin to the extent of 10 and 30 per cent respectively, the B_2 group are quite unaltered by such treatment, or by condensing and drying.

Vitamin C (Ascorbic Acid).—Scurvy, a disease known in very early times amongst mariners and explorers who were obliged to subsist for long periods on highly artificial diets, is now known to be due to lack of ascorbic acid. The symptoms of acute scurvy are a weakness of the blood capillaries and a consequent tendency for them to rupture, causing haemorrhage. Lack of ascorbic acid also affects dentition. Harris[1] considers that sub-scurvy may arise even in adults when the intake of ascorbic acid is not high enough. It would seem that this is another of those substances needed to maintain the body in full vigour. As long ago as 1593 Sir Richard Hawkins mentioned that to his own personal knowledge scurvy had accounted for the loss of 10,000 seamen. He also placed on record the great recuperative qualities of oranges and lemons.

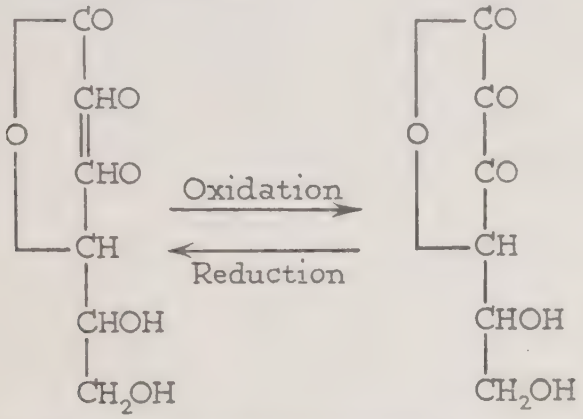
The quantity of ascorbic acid in milk is of the order of approximately 1–2 mg. per cent as contrasted with 20–40 mg. per cent in cabbage and 150–200 mg. per cent in black currants. This small quantity is further reduced by oxidation and exposure to light. In human nutrition vegetables and fruits supply the bulk of our ascorbic acid requirements. On account of the ease with which ascorbic acid undergoes oxidation, the processing of milk is bound to cause losses. Pasteurisation causes a 20 per cent loss, sterilisation 50 per cent, and drying 20–30 per cent. Losses in making evaporated milk amount to 60 per cent, although they are much less severe in the making of sweetened condensed milk (15 per cent).

The removal of ascorbic acid from milk is accelerated by the presence of traces of copper and by visible light in the presence of dissolved oxygen. Ultra-violet light, in conjunction with riboflavin, is also a potent accelerator. Noll and Supplee[2] found that

[1] Harris, *The Vitamins in Theory and Practice.*
[2] Noll and Supplee, 1941, *J. Dairy Sci.*, 24, 993.

when milk is de-aerated the ascorbic acid is not adversely affected by light, traces of copper or even sterilisation. These factors are apparently ineffective without dissolved oxygen.

The ease with which ascorbic acid suffers oxidation, and the known reversibility of the reaction suggest that, like the B group, one of the biological functions of this compound is to assist in oxidation-reduction reactions.



Vitamin. D—The chemical relationship between this vitamin and the sterols has been discussed on page 27. Its prime function is to facilitate the retention of calcium and phosphorus absorbed from the alimentary canal. The blood needs a supply of these elements not only for the purpose of bone formation, but for general maintenance purposes also. Acute vitamin D deficiency causes rickets, but moderate deficiencies are considered to have bad effects on health and resistance to disease. Although malformed limbs due to faulty hardening of the bone tissue are common symptoms of rickets, the effects of the disease are far-reaching.

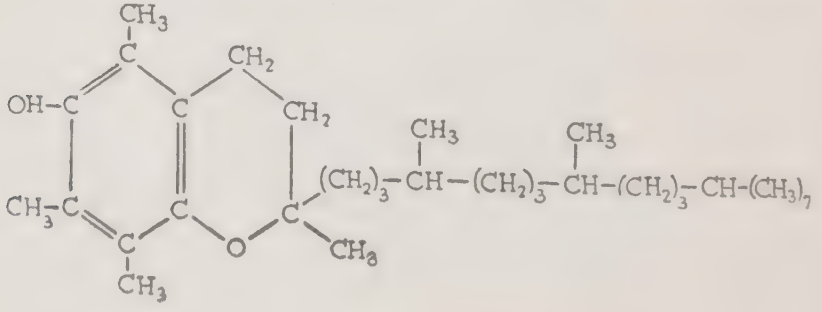
Ultra-violet light may produce vitamin D by acting on sterols present in the epidermal cells of animals and thus augments the supply of this vitamin in the food. For this reason the quantity of vitamin D in milk is more closely related to the hours of sunshine enjoyed by the cows than to feeding. For the same reason also in this country there is about four times as much in summer milk as there is in winter-produced milk. Good sources of vitamin D are fish-liver oils, egg yolk, and 'synthetic' preparations of irradiated sterols, such as dehydro cholesterol and ergosterol. Even in normal times there are very few foods of the human diet which are rich in this vitamin, and in times of war special measures must be taken to avoid the evil consequences

of a lack of it, particularly during the winter. Preparations of vitamins A and D are used extensively in infant-feeding and in the fortification of margarine.

During the years prior to the Second World War, Australian and New Zealand butters came on the English market with the widely advertised claim that they were far superior in the sunshine vitamins A and D than the normal winter-produced English butter. There was much to justify the claim. Vitaminised margarine may also claim that with a steady all-the-year-round level of these two vitamins, it is on this account superior to winter butter with its very variable vitamin potency. It seems likely that after the present Second World War, English butter will have to face serious competition on its vitamin merits. While the vitamin A and carotene content of winter-produced milk may be much improved by better feeding, it is much more difficult to raise the winter level of vitamin D. However, by feeding irradiated yeast a ten- or twenty-fold increase in vitamin D content may be secured. Also, exposure of milk to the light of a carbon arc lamp or a quartz mercury lamp brings about a great improvement in vitamin D content. The production of irradiated milk needs careful control, as, for reasons already stated, too long an exposure may lead to the production of off-flavours. The intensity of illumination, rate of milk flow, and thickness of the exposed film are other factors which have to be controlled. Milk and butter could be enriched by preparations of vitamins A and D, but as special legislation would be necessary, the problem would have to receive a thorough examination in all its aspects.

It is certainly some consolation to learn that the small amounts of vitamin D which milk contains are not adversely affected by pasteurisation, sterilisation, condensing, or drying.

Vitamin E or α Tocopherol.—This fat soluble vitamin was found necessary to prevent sterility in rats. Rich sources are



wheat germ oil, and green leaves, but it is also present in a wide variety of foods, including milk and meat.

The following table gives a general summary of the distribution of the common vitamins in milk and dairy products.

	A	B_1	B	C	D
Raw whole milk	Fair–Good	Very fair	Good	Very fair	Very fair
Pasteurised milk	Fair–Good	Slight	Good	Slight	Very fair
Sterilised milk	Fair–Good	Very slight	Good	Very slight	Very fair
Dried whole milk	Good	Slight	Good	Slight	Fair
Evaporated whole milk	Good	Very slight	Good	Very slight	Fair
Condensed whole milk sweetened	Good	Slight	Good	Slight	Fair
Separated milk, Buttermilk, Whey	Absent	Very fair	Good	Very fair	Absent
Cream	Good	Slight	Slight	Slight	Fair
Butter	Good	—	—	—	Fair
Cheese	Good	Slight	Very fair	—	Fair

Gases Present in Milk.—As drawn from the udder, milk contains about 8 per cent by volume of dissolved gases among which carbon dioxide predominates. In contact with the atmosphere this amount falls to approximately 6 per cent, but the change is not merely one of quantity. There is a significant alteration of composition as the milk gases reach equilibrium with those of the atmosphere. Carbon dioxide is lost and oxygen and nitrogen gained, as the figures in the table will show.

CHANGES IN COMPOSITION OF DISSOLVED GASES AS MILK REACHES EQUILIBRIUM WITH THE ATMOSPHERE

	Oxygen	Nitrogen	Carbon dioxide	Total
	Per cent	Per cent	Per cent	Per cent
Milk from the udder[1]	0·092	1·184	6·577	7·853
Milk from commercial plant reservoir[2]	0·47	1·29	4·45	6·21

It is of interest to note that the equilibrium concentration of oxygen and nitrogen is of the same order as the solubility of these gases in distilled water.

[1] Frayer quoted by [2] Noll and Supplee, 1941, *J. Dairy Sci.*, 24, 993.

In view of the importance of dissolved oxygen in fat oxidation and ascorbic acid removal, it is worthy of note that the quantity may be reduced to negligible proportions by a combination of heat and vacuum treatment. When this is done the ascorbic acid is able to withstand the normal oxidative influences.

Sulphuretted hydrogen and its derivatives may be present in heated milk owing to protein decomposition. Further, it is well known that milk is able to absorb the odours of silage, turnips, etc., and that a number of reported taints due to feeding these foods may be, in effect, due to this remarkable absorptive capacity of milk.

CHAPTER II

MILK ACIDITY AND SOME PHYSICAL PROPERTIES OF MILK

Milk Acidity.—The hydrogen ion concentration of milk is approximately $10^{-6.6}$ gms. per litre (i.e. its *p*H is approximately 6·6). This concentration lies on the acid side of the neutral *p*H 7, and milk is therefore able to unite with sodium hydroxide and other alkalis. So it happens that milk fresh drawn from the udder possesses a certain acidity which may be termed *natural acidity* to distinguish it from *developed acidity* in the form of lactic acid. If 10 ml. fresh drawn milk are titrated with N/9 sodium hydroxide, using phenolphthalein as an indicator, an average of about 1·6 ml. of alkali will be required. This, expressed in terms of lactic acid in the customary manner, corresponds to 0·16 per cent. This is quite a normal property of milk, being caused by casein, acid phosphates, and citrates, and to a lesser degree to albumin, globulin, and carbon dioxide. Natural acidity, like the *p*H, varies from one cow to another. Caulfield and Riddell[1] examined the natural acidity of 811 samples of milk, excluding that of early and late lactation, and found variations for individual samples from 0·08–0·30 per cent expressed as lactic acid. Average values for four breeds were: Ayrshire 0·16 per cent, Holstein 0·16 per cent, Guernsey 0·17 per cent, and Jersey 0·18 per cent. In colostrum the natural acidity is high, being due in part to its very high protein content. In early lactation also the value is above average, but it falls to normal about the second month, thereafter remaining fairly steady until the last month of lactation when a further decline occurs. Being subject to such individual variation and lactation influences, it is clear that no general assessment of natural acidity is possible. In a study of the mixed milk of the College herd the author found that 80 per cent of the natural acidity was shared equally between casein and the serum constituents (acid phosphates and citrates, serum proteins and carbon dioxide). The remaining 20 per cent was due to secondary reactions taking place during the actual titration.[2]

[1] Caulfield and Riddell, 1936, *J. Dairy Sci.*, 19, 235.
[2] Ling, 1937, *J. Dairy Res.*, 8, 173.

Several important practical considerations emerge from an appreciation of the foregoing observations. One of them refers to the common dairy practice of using titratable acidity to express lactic acid. Whenever milk is titrated with a standard alkali against phenolphthalein as indicator, the titration value is the sum of natural plus developed acidity.

Titration value of milk = natural acidity + developed acidity.

For reasons already stated it is not possible to accept a general average figure for natural acidity, and there is therefore no means of converting the total titration value into terms of lactic acid. In dairy practice it is customary to express the total acidity as lactic acid, but the student is obliged to note that however convenient such a procedure might be, the chemical objections are undeniable. The titration value merely represents the amount of standard alkali required to shift the *p*H of the sample to about 8·4 at which the change of colour of the indicator is perceptible. As yet there is no simple routine test for estimating lactic acid in milk and dairy products.

Because of these drawbacks to titratable acidity it has been suggested by several workers that in cheese-making one should be guided by *p*H values rather than titration values. On theoretical grounds this view has much to commend it, but at the time of writing there is no weight of evidence to show that cheese-making is more efficiently controlled by *p*H estimations. No doubt both methods will ultimately come into use since neither is entirely satisfactory when used alone.

Another matter of considerable importance is the use of titratable acidity as a rapid platform test for rejecting milks unsuitable for converting into evaporated milk. Because developed acidity has such a powerful influence in lowering the temperature of heat coagulation of casein, it becomes a matter of vital necessity to reject milks which are likely to cause trouble on this score. There being no convenient platform test to estimate lactic acid in milk, the condenseries are obliged to rely upon observations of smell and taste coupled with titratable acidity. An arbitrary maximum of 0·18–0·19 per cent is taken as the dividing line between satisfactory and unsatisfactory deliveries. Pending the discovery of a more direct method, this procedure appears to be a workable compromise. This does not, however, mitigate the injustice inflicted upon a producer whose herd or

occasion gives milk of high natural acidity, and on that account fails to pass the platform test.

Milks of high natural acidity are usually high in their total solids content. The constituents which cause the milk to take up more than the normal amount of sodium hydroxide are those which possess a fair buffer capacity. This phenomenon was touched upon in the general treatment of proteins (page 33). It may be stated again that substances like proteins, phosphates, and citrates are able to resist changes of *p*H which would normally occur when acids or alkalis are added to equivalent volumes of distilled water. They act as dampers whose function is to maintain the *status quo* so far as lies in their power. Milks of high natural acidity are therefore more highly buffered than is normally the case. So it happens that in such milks more lactic acid must develop before the *p*H reaches the iso-electric point of casein (4·6) which means that a longer time will be required before such samples curdle at ordinary temperatures. It is a common observation that milks of high natural acidity when compared with low natural acidity milks of equal hygienic status, show a much longer keeping quality,

Practical details relating to the acidity test will be found in Volume 2, and the employment of the test in cheese-making is discussed on page 155.

Some Physical Properties of Milk.

Colour and Flavour.—The characteristic white opalescence of milk is due to a scattering of light by the colloidal particals. The yellow colour of carotene being confined to the fat phase becomes apparent in the cream layer and is further concentrated in butter. Addition to milk of dilute acid or rennet will precipitate casein and fat, and the whey which separates will be seen to possess a distinct green-yellow colour due to the pigment riboflavin. There is also a faint blue opalescence visible in separated milk and low fat milks, the origin of which is at present obscure.

Flavour is a property difficult to define, but there is no doubt that taste and smell are used in its assessment. Just as there are primary and pastel shades, so there are primary tastes and odours and more delicate flavours produced by the blending of two or more primary characters. There are some individuals whose perception of the delicate flavours of milk and dairy products is dull, just as there are some who are unable to appreciate

certain pastel shades. Because of these individual differences much of the published work on milk flavours appears contradictory.

The sweet taste of lactose is balanced against the salty taste of chlorides, and both are damped down by proteins. Some workers attribute the characteristic rich flavour of dairy products to the phospholipids. The flavour of milk is thus a delicately balanced property. As lactation advances, lactose declines while chlorides increase, so that the balance is deflected towards 'salty.' A similar dislocation is caused by udder disturbances. Roadhouse and Henderson[1] describe how the flavour scores of twenty-four individual samples of milk ran parallel to the lactose/chloride ratio.

The normal balance of flavour may also suffer through the passage into milk of flavouring essences present in such plants as thyme, mint, camomile, or garlic. The following plants when consumed by the cow are said to cause peculiar taints—ox-eye daisy, buttercup, dog's mercury, tansy, marsh marigold, yarrow, ivy, sun spurge, and garlic mustard. Whether odoriferous foods like-silage are able to cause off flavours by their passage into milk from the blood or by the exercise of that remarkable absorptive capacity of milk for any such vapours present in the atmosphere, is still a matter for debate. Both possibilities are avoided by feeding such foods immediately after milking. If the former possibility exists the tainting material may be excreted before the next milking, and if the latter, all traces of the food will have been removed so that no odours are present during milking. Lucerne hay has been the subject of much attention in this connection. Several workers describe a flavour which they call 'feedy', and are able to trace it to the lucerne hay of the ration. Again the taint was avoided by feeding immediately after milking, the evidence in this case pointing to the flavour having passed into milk through the blood.[2] Similar precautionary measures were found effective in the case of fishy taints caused by the feeding of molassed beet by-products (page 23).

Reference has been made to rancid and oily taints (pages 14–16 respectively). In the latter case the catalytic action of copper was discussed. Quite apart from its catalytic action it is considered by Davies[3] that the presence of three parts per million of copper will impart a distinctly metallic taste.

[1] Roadhouse and Henderson, 1941, *The Market Milk Industry*, p. 177.
[2] Ibid., p. 181.
[3] Davies, 1939, *The Chemistry of Milk*, p. 294.

Although when freshly drawn, milk has a certain 'cowy' odour, this passes off and by the time it reaches the consumer there may be little or no odour. As lactic acid develops, the flavour of milk changes in a very definite manner, moving continuously towards the characteristic sour flavour. This is the resultant of lactic acid and the various by-products, such as butyric acid, diacetyl, etc. Owing to the simultaneous production of odoriferous by-products, milk possessing an odour is commonly assumed to be in process of souring. The personal factor in the detection of taints is so very variable that observations of taste and smell must always be supplemented by more measurable criteria.

The Specific Gravity of Milk.—The average specific gravity of milk at 15·5°C. (60°F.) is 1·032, but this is one of its most variable properties. For individual samples the variation is very wide, namely, 1·0135–1·0397, and on this account the range for mixed milk will depend very largely on the size of the herd. Indeed, as the expression 'mixed milk' may be used to describe the bulk milk of five or fifty-five cows, it is so vague as to be almost meaningless. Thus while Davies[1] states that mixed milk may range from 1·028–1·034, Elsdon and Walker[2] maintain that the mixed milk of a herd rarely falls outside the limits of 1·030–1·034. Such variations merely reflect the fluctuating proportions of water, fat, and non-fatty solids, the specific gravities of which are 1, 0·94, and 1·62 respectively. Further, since both fat and non-fatty solids are themselves mixtures of varying composition, their own specific gravities will be subject to some slight variation. Again there will be differences between the specific gravities of solid fat and liquid fat, even although both may be at the same temperature. The student is reminded that one of the peculiarities of milk fat is the appreciable time-lag in adjusting its physical condition to a change of temperature (refer back to page 10). These observations are made in order that the student should be able to appreciate the limitations of commonly used formulæ connecting the specific gravity of milk with its chemical composition. Several such formulae have been calculated from time to time, and of these, Richmond's has proved most convenient.

$$\text{T.S.} = \text{L.R.}/4 + 1{\cdot}2\text{F} + 0{\cdot}14$$

T. S. = % total solids, L.R. = lactometer reading at 60°F. i.e. 1000 (S.G. minus 1)
F = % fat.

[1] Davies, 1939, *The Chemistry of Milk*, p. 280.
[2] Elsdon and Walker, 1942, Richmond's *Dairy Chemistry*, p. 81.

More recently Richmond's formula has been recalculated for use with the modern improved density hydrometer.

$$T.S. = DH/4 + 1{\cdot}21\,F + 0.66$$

where T.S. = % total solids, D.H. = density hydrometer reading at 20°C. (i.e. 1000 × density—1)
F. = % fat.

These are very convenient and much used approximations, but the assumption throughout is that the sample contains the normal constituents of average specific gravity and in the normal proportions. It will be seen later that there are occasions when it is by no means certain where normality ends and abnormality begins.

Addition of water will lower the specific gravity of milk, but as milk of high fat content also has a low density, it is clear that this value alone cannot be used to detect added water. Owing to the removal of fat, separated milk has a higher specific gravity (about 1·036). It would therefore be quite possible to water milk and restore its original specific gravity by addition of separated milk. Such a procedure would contravene the sale of Foods and Drugs Act, and is mentioned solely for the purpose of illustrating the inadequacy of specific gravity tests alone as criteria of the genuineness of milk. The test is rapid and convenient and finds its uses in laboratory routine. The specific gravity of milk is lowered by addition of water, addition of cream, or by increased temperature, while the contrary effect is caused by addition of separated milk, removal of fat, or reduction of temperature.

A matter of great interest is the phenomenon discovered by Recknagel and known by his name. He observed that the specific gravity of fresh-drawn milk is lower than is indicated an hour or so later. The difference is of the order 0·001, i.e. one lactometer degree. Recknagel found that the time required for the completion of this change could be shortened by keeping the sample cold, and he came to the conclusion that changes in casein were responsible. Increases of specific gravity had been observed up to some twenty-two hours after milking.

From his own experiments, Richmond was led to the conclusion that the true explanation lay in the slow conversion of liquid fat to the solid condition. The significance of Richmond's interpretation of the phenomenon lies in the fact that, if true, the change should be reversible and thus would be liable to recur whenever there is an alteration in the physical state of the fat. If, however, the original explanation is the more correct, there

should be no further change once the sample has aged; unless the changes in casein are of a reversible nature.

The subject has been regarded as one of academic or historic interest and for many years was neglected. It has recently come into prominence again through the work of Boden and Campbell,[1] who found that the new density formula gave lower values for non-fatty solids than the old lactometer formula of Richmond. The discrepancy is of the order 0·2 per cent. Richmond's formula was put forward for samples in which the Recknagel phenomenon was complete, whereas the modern formula is used for milks warmed to 40°C. and then cooled to 20°C.[2] in which the phenomenon would be absent, since presumably the fat would be liquid. The modern density hydrometer formula is calculated from Richmond's with no correction for the Recknagel change, and Boden and Campbell suggest this as one reason for the observed discrepancies. This is a practical subject of great importance, and it may be necessary to amend either the modern formula or the technique as at present prescribed.

That changes in the physical condition of the fat are partly responsible for the Recknagel phenomenon there can be little doubt; but the work of Pyenson and Dahle[3] on bound water in milk and dairy products leads to the conclusion that, as originally suggested, changes in casein are also partly responsible.

Bound Water.—Water is present in milk to the extent of about 87·5 per cent, but it is not all in the free condition. Some is firmly bound by the milk proteins, some by phospholipids, and some by the substances adsorbed on the fat globules. The quantities bound in milk and some dairy products are shown in the following table.[4]

Product	Age in hours	Total water %	Water bound per 100 gms. of product
Raw milk . .	24	86·75	3·18 gms.
Skim milk . .	24	90·56	2·13 ,,
Cream 1 . .	8	70·92	2·5 ,,
,, 2 . .	8	56·80	3·42 ,,
Buttermilk . .	—	91·75	1·75 ,,
Colostrum . .	24	80·83	4·65 ,,

[1] Boden and Campbell, 1942, *J. Dairy Res.*, 13, 45.
[2] *British Standards Institution, Publication No. 784.*
[3] Pyenson and Dahle, 1938, *J. Dairy Sci.*, 21, 601.
[4] Ibid., 21, 169, 407, 601.

Of the 3·18 gms. of bound water in 100 gms. of milk, 50 per cent is held by casein, 30 per cent by albumin and globulin, and about 15 per cent by the fat globule membranes. These figures are rather misleading owing to the very different quantities of these substances in milk. Calculated as water bound per gramme, the figures are 6 gms. for the phospholipids, 1·3 gms. for albumin and globulin, and 0·7 gm. for casein.

When milk is stored at 40°F. the bound water slowly increases until an equilibrium is established.

$$\text{Bound water} \rightleftarrows \text{Free water}$$

As these increases occur simultaneously with the Recknagel effect Pyenson and Dahle suggest that they are in part responsible for the latter phenomenon. This view is strongly supported by the fact that separated milk shows both phenomena. The following table illustrates some of the results of this valuable research.

Product	Aged at 40°F.	Specific gravity at 60°F.	% Bound water
Raw milk . .	0 hours	1·0314	2·79
	8 ,,	1·0328	2·98
	24 ,,	1·0332	3·18
Raw skim milk .	0 ,,	1·0359	1·81
	8 ,,	1·0366	2·05
	24 ,,	1·0371	2·13

The equilibrium thus attained may be disturbed by heating above 160°F. when after cooling and ageing the samples were found to contain less bound water.

The whole subject is one of great scientific interest, and in the author's opinion, a further exploration may serve to elucidate a number of hitherto obscure problems.

Surface Tension.—Compared with water, the surface tension of milk is low. The actual ratio of the two values is 5 : 2. Somewhat higher values are shown by separated milk, while cream has a lower surface tension. The colloidal constituents, particularly the proteins, are responsible for this lowering of surface tension. One characteristic of such colloidal solutions is a strong tendency for the substances which lower the surface tension to be concentrated at the liquid/air interface. When milk is warmed calcium caseinate gathers at the liquid air surface, together with smaller amounts of albumin, globulin, and fat. These constitute the

familiar skin. If milk is agitated, a similar precipitation occurs around the air bubbles and the phenomenon of frothing results.

Warming above 30°C. causes a further reduction of surface tension, and there is a marked tendency to foam on account of the more active concentration of protein around the air bubbles. The protein films being somewhat elastic confer a certain measure of stability to the foam which ultimately collapses, leaving the disrupted films on the surface of the liquid. Although the temperature range, 20–30°C., represents the minimum foaming conditions, milk cooled below 20°C. shows a further increase in foaming properties. In this case the foam is not so stable as that obtained above 30°C., and its physical nature is different. This cannot be explained on a surface tension basis as in the previous case. No doubt other factors, such as increased hydration of proteins, and change in condition of the fat, come into play at lower temperatures. Thus, while surface tension effects may partly explain the foaming qualities of milk, they are clearly not the only factors involved.

Viscosity.—Milk is considerably more viscous than water, mainly on account of the fat emulsion and colloidal particles. Therefore alterations in the physical nature of the fat and changes in the hydration of proteins are bound to have some effect on viscosity. Similarly, alterations in the state of division of any dispersed constituents, such as occurs when the fat globules are greatly subdivided by homogenisation, will also have their effect. Homogenisation increases viscosity. This property of milk assumes greater importance in considering both the formation and properties of cream. Whether the fat is separated mechanically or by the force of gravity, the viscosity of the surrounding liquid resists the movement of the globules. As a viscous cream creates the impression of 'richness' this property will receive further consideration under the appropriate heading (pages 125–127).

Specific Heat.—From the separate specific heats of the milk constituents and a knowledge of their respective percentages, the specific heat of the mixture may be calculated. Taking values of specific heat as 0·5 for fat, 0·3 for lactose, 0·5 for protein, 0·7 for ash, and 1·0 for water, the resultant for milk of normal composition is 0·93. This approximates to the observed specific heat of warm milk in which the fat is liquid but is somewhat lower than the values obtained below 19°C., because some of the heat supplied

to milk at a temperature near the melting-point of the fat is used to supply the latter with its latent heat of fusion. On this account the temperature-specific heat curves for milk or cream show 'humps' with maxima at 19°C., whereas whey or separated milk show no such maxima. At 60°F., the following specific heats are observed: milk 0·94, separated milk 0·94, whey 0·98, butter 0·53, cream of 30 per cent fat content 0·98, 60 per cent fat cream 1·05, and cheese 0·64. At 40°C. the values for milk, 30 per cent fat cream, and 60 per cent fat cream fall to 0·93, 0·85, and 0·72 respectively.

Refractive Index, Boiling-point and Freezing-point.—These three characteristics are more directly related to the soluble constituents of milk, the colloidal matter making very little contribution to them.

Refractive Index.—Milk has a refractive index of about 1·35, that of water being 1·33. Addition of water would therefore lower the refractive index of milk, but as considerable variation is observed in the values for genuine milk, it is not possible to use this property as a criterion of the genuineness of milk samples. Some workers have used the immersion refractometer with sera obtained by precipitating fat and colloidal matter from milk by addition of copper sulphate solution. This instrument gives a reading which is a measure of the refractive index, and the test has been used as a rapid means of sorting suspected samples for further examination. The general experience has been that the test has not fulfilled the high hopes which were originally centred upon it. Indeed, it has little advantage over the routine estimations of fat and non-fatty solids.

Boiling-point.—A solution boils at a higher temperature than does the pure solvent according to the concentration of the dissolved substance. Here again the milk constituents in true solution are mainly responsible for the elevation above 100°C. of the boiling-point. Davies[1] calculates that the boiling-point of milk should be 100·15°C., but actual observation proves this to be under-estimated by about 0·40°C. One reason for this discrepancy is undoubtedly an alteration of the normal ionic⇌molecular⇌colloidal equilibrium as a result of heating. Addition of water lowers the concentration of the dissolved substances responsible for the elevation of boiling-point, and it might be expected that a boiling-point determination would serve to

[1] W. L. Davies, 1939, *The Chemistry of Milk*, p. 292.

detect this particular form of adulteration. There are, however, many practical difficulties in finding the true boiling-point of milk, and hitherto the method has not found application in this direction.

Freezing-point.—A solution freezes at a lower temperature than the pure solvent, the depression of freezing-point depending upon the concentration of the dissolved substance. Further, this freezing-point depression is a function of the osmotic pressure of the solution. On physiological grounds it is reasonable to believe that the osmotic pressure of milk would be approximately the same as that of the blood, which is a fairly constant value. The freezing-point of milk is, in fact, its most constant property, and as addition of water is bound to alter the normal value, it is clear that freezing-point estimations should prove valuable in detecting this form of adulteration. Using the Hortvet apparatus and technique, Elsdon and Stubbs found the mean value of the freezing-points of 330 samples of milk to be $-0{\cdot}545$°C.[1] In order to obviate the necessity for the negative sign, it is customary to refer to the mean freezing-point *depression* as being 0·545°C. Addition of water reduces this value and it is usual to regard a freezing-point depression of less than 0·53 as indicative of added water. This aspect of the subject will be dealt with in greater detail in another section (page 97), but it is necessary to add that as separated milk has the same freezing-point as milk, the method cannot detect adulteration by this means. Also, by bringing more mineral matter into true solution, developed acidity increases the osmotic pressure and thus gives a greater freezing-point depression.

[1] Elsdon and Stubbs, 1934, *Analyst*, 59, 146.

CHAPTER III

THE COMPOSITION OF MILK

Previous chapters have dealt primarily with the qualitative aspect of milk composition, but on several occasions it became necessary to refer to the variations in concentration shown by certain constituents. These variations make it difficult to assess the quantitative composition of milk. There is only one approach to the problem, and that is to analyse large numbers of samples taken from different herds of different breeds in various parts of the country and over different periods of the year. When the results are converted to mean values an 'average' composition is obtained. By this means factors such as individuality, breed, stage of lactation, season, etc., which are known to influence the composition of milk, are damped down or practically eliminated. Even so, the question might be raised, "*Of what use is this average when most practical problems are concerned with the milk of localised herds of perhaps ten to forty cows?*" It is often emphasised that the 'average' man is non-existent. Whether this contention might apply to milk is a matter which the author hopes will become apparent in the treatment which follows.

Regarding the number of samples necessary to enable an average to be calculated, no hard and fast rule can be laid down, but most investigators have worked on a minimum of some 700. Some have taken individual samples, while others have taken bulk samples with no record of the number of cows in the herds. The problem of giving published data their correct assessment may be illustrated by the following example. An investigator 'A' analyses 1,500 samples of mixed milk from one herd of twenty cows, and another, 'B,' takes 15 samples from each of 100 different herds, none of which contains less than 10 cows. Both have analysed 1,500 samples of mixed milk, but are their results comparable? In the author's opinion they are not. Investigator 'A' spreads his work over more than two years and his results refer to a very varied set of seasonal conditions, whereas 'B' is able to complete his work in a much shorter time. Another important difference lies in the fact that 'B' has obtained his

results from very many more cows. Both have their intrinsic merits. The results obtained by 'B' would give a more valuable estimate of the average composition of milk, but in many respects the work of 'A' is of greater practical value in demonstrating day-to-day fluctuations in composition, lactation effects, seasonal variations, and general management factors, such as are commonly manifest in a herd. Both types of investigation will be encountered in a search through the literature of the subject. In this matter the student must acquire the habit of critical reading, or at least should be able to exercise discrimination so as to avoid coming to conclusions which the investigators never intended.

Average values alone are of very limited use. In an investigation into the composition of milk, Cranfield, Griffiths, and Ling[1] examined 732 samples of mixed milk from 15 herds, corresponding to some 418 cows. The average percentages of fat and non-fatty solids were 3·71 and 8·75 respectively. But much more information was required. At the time of the investigation questions were being asked such as: "*What is the extent of variation on either side of the mean composition of genuine milk?*" or "*What is the likelihood of genuine milk falling down the legal presumptive limits of* 3·0 *per cent fat and* 8·5 *per cent non-fatty solids?*" The mere statement of average values is not enough ; the distribution of the results each side of the mean values being of equal or perhaps greater importance. From the distribution of the fat percentages it will be seen that although the mean was 3·71, there were sixty instances out of 732 (i.e. 8·2 per cent) in which the values fell below the 3 per cent limit, and that more than 4 per cent fat was shown in 205 instances (i.e. 28 per cent of total).

[1] Cranfield, Griffiths, and Ling, 1927, *J. Agric. Sci.*, 17, 62.

FREQUENCY DISTRIBUTION OF THE PERCENTAGES OF FAT AND S.n.F. IN THE MIXED MILK OF FIFTEEN HERDS

FAT		S.n.F.	
Range per cent	No. of samples	Range per cent	No. of samples
2·20–2·39	3	7·90–7·99	1
2·40–2·59	8	8·00–8·09	3
2·60–2·79	12	8·10–8·19	5
2·80–2·99	37	8·20–8·29	13
3·00–3·19	67	8·30–8·39	26
3·20–3·39	96	8·40–8·49	59
3·40–3·59	99	8·50–8·59	103
3·60–3·79	112	8·60–8·69	107
3·80–3·99	93	8·70–8·79	113
4·00–4·19	83	8·80–8·89	116
4·20–4·39	44	8·90–8·99	80
4·40–4·59	31	9·00–9·09	47
4·60–4·79	21	9·10–9·19	33
4·80–4·99	7	9·20–9·29	17
5·00–5·19	10	9·30–9·39	7
5·20–5·39	4	9·40–9·49	1
5·40–5·59	3	9·50–9·59	1
5·60–5·79	1	—	—
5·80–5·99	1	—	—
	732		732
Mean % of fat, 3·71		Mean % of S.n.F., 8·746	

Another way of conveying information regarding the extent of variation around the mean values is to calculate what is known statistically as the standard variation. This value gives the range each side of the mean into which two-thirds of the observations may be expected to fall. In the above investigation the mean fat percentage was 3·71 with a standard deviation of $\pm 0{\cdot}56$, meaning that two-thirds of the observations may be expected to lie between 3·15 and 4·27 per cent fat. Thus the statement of mean together with the standard deviation is of far greater value than average figures, even when the latter are accompanied by the extremes of variation, as was the custom in earlier work on the subject. The following table summarises some of the more extensive researches. Wherever possible the standard deviations are given.

Author	No. of samples	No. of herds	No. of cows	Fat % Average	Fat % Max.	Fat % Min.	Fat % S.D.	Solids-not-fat % Average	Solids-not-fat % Max.	Solids-not-fat % Min.	Solids-not-fat % S.D.
1. Richmond . .	330,000	—	—	3·78	—	—	—	8·74	—	—	—
2. Crowther . .	2,800 M	49	209	3·70	5·3	2·0	—	—	—	—	—
	1,420 M	6	78	—	—	—	—	8·78	9·5	8·4	—
3. Cranfield, Griffiths, and Ling . .	732 M	15	418	3·71	6·0	2·2	±0·56	8·75	9·6	7·9	±·24
4. Elsdon *et al.* appeal samples . .	1,854	—	—	3·47	—	—	—	8·82	—	—	—
All samples . .	771,662	—	—	3·61	—	—	—	8·77	—	—	—
5. Baker and Cranfield.	330,000 M	—	—	3·61	—	—	—	8·91	—	—	—
6. Tocher . . .	676 I	—	676	3·95	7·5	1·66	±0·78	8·80	10·66	7·0	±0·44
7. Golding *et al.* . .	3,115	1	10	3·89	5·17	2·6	—	8·89	9·28	8·4	—
8. Golding *et al.* . .	1,498	1	10	3·82	4·94	2·62	—	8·81	9·19	*8·06	—
9. Van Slyke . .	5,552	—	—	3·9	—	—	—	9·0	—	—	—
10.—Burr . . .	500,000 M	—	—	—	—	—	—	8·76	9·25	8·25	—†

M = Mixed milk. I = Individual samples where stated by authors. S.D. = Standard deviation.
* Conditions of Drought. † 96 per cent of samples were within this range.

[1] Richmond's *Dairy Chemistry*, Elsdon and Walker, 1942, p. 9.
[2] Crowther, 1917, *J. Agric. Sci.*, 1, 149.
[3] Cranfield, Griffiths, and Ling, 1927, *J. Agric. Sci.*, 17, 62.
[4] Richmond's *Dairy Chemistry*, Elsdon and Walker, 1942, pp. 6 and 9.
[5] Baker and Cranfield, 1933, *J. Dairy Res.*, 4, 246.
[6] Tocher, *Variations in the Composition of Milk*, 1925, H.M. Stationery Office.
[7] Golding *et al.*, 1932, *J. Dairy Res.*, 5, 48.
[8] Golding *et al.*, 1935, ibid., 6.6.
[7] and [8] as quoted by Davies, *The Chemistry of Milk*, 1939, p. 15, 17.
[9] Van Slyke, *Modern Methods of Testing Milk and Milk Products*, 1927.
[10] Quoted by Mackintosh, 1940, *J. Roy. Agric. Soc. England*, 101, 78.

THE DETAILED COMPOSITION OF MILK

A SUMMARY OF SOME EXTENSIVE RESEARCHES

	Rich-mond[1]	Elsdon[2]	Cranfield, Griffiths, Ling[3]		Tocher[4]		Van Slyke[5]	Davies *et al* A.M. milk	
	%	%	%	S.D.	%	S.D.	%	%	
Water . .	87·34	87·25	87·54		87·19		87·1	87·84	S.D
Fat . .	3·75	3·75	3·71	±·56	3·95	±·78	3·9	3·28	±·2
Protein . .	3·40	3·20	3·25	±·18	3·17		3·2	3·00	±·1
Other nitrogenous substances .	—	0·20	—		0·35		—	0·20	
Lactose . .	4·70	4·70	4·74	(diff.)	4·64	±·37	5·1	4·87	±·16
Ash . . .	0·75	0·75	0·76	±·025	0·70	±·05	0·7	—	
Other substances	0·06	0·15							
Total . .	100·00	100·00	100·00		100·00		100·0	—	
P_2O_5 . .	0·22	—	0·234	±·018	—		—	—	
CaO . .	0·15	—	0·184	±·013	—		—	—	

S.D. = Standard deviation.

With regard to the nitrogen distribution of milk, Rowland has used an improved technique for estimating the three proteins and the proteose peptone, from which it would appear that earlier analyses failed to effect quantitative separation of these various fractions. His own work indicates that normal milk containing 3·18 per cent true protein would give 2·63 per cent casein, 0·31 per cent albumin, 0·11 per cent globulin, and 0·13 per cent proteose peptone.[7] In addition, some 5 per cent of total nitrogen is contained in non-protein matter for which no conversion factor is at present available. It seems desirable to combine this work with one of the foregoing researches in order to present as complete a picture as possible of the composition of milk. Pending the publication of further data, the following may be accepted with reserve.

[1] Richmond's *Dairy Chemistry*, 1930, pp. 36, 37, 296.
[2] Elsdon and Walker, Richnond's *Dairy Chem.*, 1942, p. 2.
[3] Cranfield, Griffiths, and Ling, 1927, *J. Agric. Sci.*, 17, 62 and 72.
[4] Tocher, *Scottish J. Agric.*, 1926, 9, 351.
[5] Van Slyke, 1927, *Modern Methods of Testing Milk and Milk Products.*
[6] Davies *et al.*, 1935, *J. Dairy Res.*, 6, 363.
[7] Rowland, 1938, *J. Dairy Res.*, 9. 47.

	Per cent	
Water	87·54	
Fat	3·71	
Casein	2·63	Protein 3·18
Albumin	0·31	
Globulin	0·11	
Proteose peptone	0·13	
Other nitrogenous substances	0·11	
Lactose	4·70	
Ash	0·76	
	100·00	

Variations in the composition of Milk.—Before considering the various factors known to influence the composition of milk, it is well to recognise that some constituents and some physical properties show much less variation than others. These differences are clearly demonstrated in the table which gives the coefficient of variation for several constituents as calculated from the works of Tocher[1] and Cranfield, Griffiths, and Ling.[2]

Coefficients of Variation

	Tocher	Cranfield, Griffiths and Ling
Non-fatty solids	5·02	2·75
Ash	7·21	3·29
Lactose	8·01	—
Total nitrogen	12·22	5·54
Casein nitrogen	13·68	—
Fat	19·76	15·1
Albumin nitrogen	20·00	—
Yield per milking	36·53	—
Yield of fat per milking	38·44	—

Note.—The coefficient of variation $= \frac{\text{Standard deviation}}{\text{Mean}} \times 100.$

Tocher found greater variation in his 676 samples than was found in the 732 of Cranfield *et al.* because they were obtained from individual cows, whereas the latter were mixed milks. Although the two columns are not strictly comparable on this account, they show the same relative variability and, further, they emphasise the great variability of fat as compared with the other major constituents.

[1] Tocher, 1925, *Variations in the Composition of Milk*, H.M. Stationery Office.
[2] Cranfield, Griffiths, and Ling, 1927, *J. Agric. Sci.*, 17, 62, and 72.

Variation is always greater in small herds than large ones. For instance, Tocher[1] calculates the possibilities of herds of different numbers of cows giving milk below the legal limit of 8·5 per cent non-fatty solids.

No. of cows per herd . .	1	2	3	4	5	6	7	8	9	10
No. of herds which would give less than 8·5% S.n.F. (per 1,000 herds)	247	165	116	84	62	46	34	26	19	15

No. of cows per herd . .	11	12	13	14	15	16	17	18	19	20
No. of herds which would give less than 8·5% S.n.F. (per 1,000 herds)	11	8	7	5	4	3	2	2	1	1

This calculation illustrates the steadying effect of numbers of cows on the composition of milk. The same effect is observed in the extent of daily variation in composition of the milk of a herd. There is general agreement that these day-to-day variations will be more extensive in the milk of smaller herds, but there is a tendency either to exaggerate the facts or to depreciate them. The subject is of fundamental importance in connection with "appeal to the cow" samples which are taken with the object of checking samples suspected of adulteration. There is an underlying assumption that if, for example, the suspected sample is below the minimum limit of 3 per cent fat, the appeal sample drawn within the next day or so from the same cows at a corresponding milking should also show a low fat content; provided, of course, the suspected sample is genuine. It is essential, therefore, that the extent of these day-to-day variations should be examined with strictest impartiality. Fat being the most variable major constituent will show the greatest daily variation. Bartlett[2] has found that the daily fluctuations of fat content are greatest during the first month of lactation and are greater in summer than winter. Observations of daily variation in two herds of the same size are likely to show differences if one of them contains more early lactation cows than the other. This may be one cause

[1] Tocher, 1927, *Scott. J. Agric.*, 10, 201.
[2] Bartlett, 1929, *J. Agric. Sci.*, 19, 438.

of the lack of conformity amongst published works on the subject, some of which are summarised in the following tables.

DAY-TO-DAY VARIATIONS IN THE MILK OF A HERD OF 24 SHORTHORN COWS OVER A PERIOD OF 39 DAYS[1]

		% Fat						
Daily variation		0	·1	·2	·3	·4	·5	·6
No. of occasions	A.M.	5	16	8	5	4	0	0
	P.M.	3	12	8	6	5	3	1

		% Non-fatty solids									
Daily variation		0	·1	·2	·3	·4	·5	·6	·7	·8	·9
No. of occasions	A.M.	2	7	6	6	9	2	2	2	2	0
	P.M.	6	8	6	7	1	4	1	4	0	1

Cranfield,[2] from a study of 359 a.m. and 362 p.m. samples from a herd of 30–35 cows over a period of one year, observed the following variations.

DAILY VARIATIONS

		Fat		Non-fatty solids	
		Over 0·5%	Over 0·75%	Over 0·5%	Over 0·75%
Number of occasions	A.M.	2	0	0	0
	P.M.	11	3	3	2

Other observations relating to day-to-day fat variations only are summarised in the table on p. 76.

Beyond establishing the fact that these daily fluctuations do occur, the published works do not allow of any general statement on the extent and frequency of such variations. Nevertheless,

[1] *Variations in the Composition of Milk*, miscellaneous pub. No. 65, 1929, Ministry of Agriculture.

[2] Cranfield, 1929, *Farmer and Stockbreeder*, October, 21.

	No. of days of test	No. of cows		Daily variation in % fat									
				0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
Elsdon[1] and Stubbs	134	40	No. of times A.M.	not	quo	ted	6	5	2	2	0	0	0
			No. of times P.M.	not	quo	ted	4	5	2	0	0	0	0
Lauder[2] and Fagan	70	20	No. of times A.M.	not	quo	ted	0	3	1	4	0	0	0
			No. of times P.M.	not	quo	ted	2	6	2	0	0	0	0
Garforth[3] report	52	18	No. of times	not	quo	ted	0	0	0	4	2	0	1
Elsdon[4] and Stubbs	50	12	No. of times A.M.	not	quo	ted	2	2	1	1	–	–	–
			No. of times P.M.	not	quo	ted	5	2	–	–	–	–	–
Tocher[5]	21	8	No. of times A.M.	2	6	1	7	3	0	1	–	–	–
			No. of times P.M.	2	4	3	6	3	1	0	0	1	–

Elsdon and Walker[6] consider it extremely unlikely that milk containing less than 3 per cent fat, even from a small herd, would show an increase of more than 0·5 per cent fat during the next two days or less, assuming that the appeal sample is taken from a corresponding milking and that the periods of milking is the same. A considerable weight of evidence is necessary to support this claim, and until more light can be thrown on the problem, the matter must remain one of opinion.

The Individuality of the Cow.—It is a common observation that even in herds kept under ideal conditions of management and feeding there are occasionally one or two cows which persistently give milk showing some abnormality of composition. Such abnormality may be a consistently high or low percentage of fat or protein, etc., which cannot be attributed to any of the known factors which will receive treatment in this section. Because of its apparently uncontrollable nature, this factor is placed at the head of the list.

Breed.—It is well recognised that the composition of milk is strongly influenced by breed, but even to-day it is difficult to obtain all the desired information which would lead to a correct appreciation of the precise extent of this effect. There are many figures available, but some have been derived from analyses of milk

[1], [2], [3], [4] Elsdon and Walker, 1942, Richmond's *Dairy Chemistry*, p. 60.
[5] Tocher, 1927, *Analyst*, 52, 470.
[6] Elsdon and Walker, 1942, Richmond's *Dairy Chemistry*, p. 60.

from selected cows of the various breeds, while others refer to milk obtained under the abnormal conditions pertaining at shows. By far the most comprehensive figures available in this country refer to the fat content of the milk of various breeds, as determined under the Butterfat testing scheme of the Ministry of Agriculture.[1, 2] They are summarised in the table.

FAT PERCENTAGE OF THE MILK OF VARIOUS BREEDS

	Year ending October 1, 1937							Year ending Oct. 1, 1936	
	Under 3%	3–4%	4–5%	5–6%	Over 6%	No. of cows	Average %	No. of cows	Average %
Jersey . .	0	47	733	913	135	1828	5·05	1687	5·04
Guernsey .	1	329	1957	791	33	3111	4·61	2831	4·61
Ayrshire .	5	989	448	6	0	1448	3·81	1133	3·78
Lincoln Red .	0	142	38	1	0	181	3·72	174	3·62
Red Poll .	42	622	161	2	1	828	3·64	694	3·62
Shorthorn .	102	2139	361	4	0	2606	3·62	2744	3·58
Friesian .	543	2652	157	1	0	3353	3·29	3153	3·26
Others . .	2	87	87	2	0	178	3·97	191	3·92
Totals . .	695	7007	3942	1720	169	13533	3·93	12597	3·89

Breed also has its effect on the non-fatty solids of milk, but published works have usually recorded the actual percentage in the whole milk. Where different fat percentages are so frequent, it is advantageous to calculate the non-fatty solid content on a fat-free basis i.e. $\frac{\text{percentage S.n.F. in milk}}{100 - \text{percentage fat}} \times 100$.

The table on p. 78 gives the non-fatty solid content of the milk of different breeds as calculated from the works of the authors quoted.

These figures show a fair measure of agreement, and it is clear that so far as the non-fatty solids are concerned the influence of

[1] 1937, *J. Min. Agric.*, 44, 875. [2] 1938, ibid., 45, 950.

PERCENTAGE S.N.F. IN FAT-FREE MILK OF VARIOUS BREEDS.

	Drakeley[1]	Tocher[2]	Davies[3]	Road-house[4]	Vieth[5]	Overman[6]
Jersey	9·82	10·1	10·1	10·05	9·79	10·03
Guernsey	9·78	9·98	10·05	10·15	—	10·2
Kerry	9·51	—	—	—	9·44	—
Dexter	9·51	—	—	—	—	—
South Devon	9·63	—	—	—	—	—
Ayrshire	9·37	8·90	9·42	9·38	—	9·34
Red Poll	9·45	—	—	—	9·28	—
Shorthorn	9·40	9·17	9·28	9·24	9·25	—
Lincoln Red	9·35	—	—	—	—	—
Friesian	9·12	8·73	8·78	—	—	—

breed is much less than it is on fat percentage. There is, however, a tendency for high fat content to be associated with a somewhat high percentage of non-fatty solids, and vice versa (*that is when the milk of one breed is compared with that of another*). It may be argued that in the case of breeds giving milk of a somewhat low fat or solid content, a more copious yield will offset this disadvantage, and while this may be perfectly true, such milk may be expected to fall below the presumptive limits of 3 per cent fat and 8·5 per cent S.n.F. much more frequently than that of other breeds.

Milking Intervals.—The general experience is that following a long interval there will be a greater yield of milk containing a correspondingly smaller percentage of fat, whereas milk drawn after a shorter interval is richer in fat but smaller in quantity. Attempts have been made to estimate the precise relationship between milking hours and milk composition, but the results show no convincing measure of agreement. No doubt the effect will be of different magnitude according to the individuality of the cow, etc. The effect of this factor is felt by the fat rather than the non-fatty solids, although reference to published work may give the impression that the latter were also involved. If a long night interval results in a lower fat content, the percentage of non-fatty solids will show a slight increase, because in a mixture of three components, if the percentage of one is reduced, there are corresponding increases in those of the remainder. The effect

[1] Drakeley, 1927 *J. Agric. Sci.*, 17, 126. [2] Tocher, 1927, *Scott. J. Agric.*, 10, 408.
[3] Davies, 1939, *The Chemistry of Milk*, p. 22.
[4] Roadhouse and Henderson, 1941, *The Market Milk Industry*, p. 12.
[5] and [6] Quoted by Elsdon and Walker, Richmond's *Dairy Chemistry*, pp. 37, 38

on the non-fatty solids is purely mathematical, and if the percentages are calculated on a fat-free basis it disappears entirely. Davies[1] illustrates these effects from data obtained at the National Institute for Research in Dairying.

COMPOSITION OF A.M. AND P.M. MILK. DAY INTERVAL 9 HOURS NIGHT INTERVAL 15 HOURS

Period	A.M. Milk			P.M. Milk			% S.n.F. on a fat free basis	
	No. of samples	% Fat	% S.n.F.	No. of samples	% Fat	% S.n.F.	A.M.	P.M.
1928–9 . .	352	3·28	8·99	337	4·69	8·84	9·30	9·28
1929–30 . .	365	3·21	8·87	365	4·55	8·84	9·16	9·26
1930–1 . .	336	3·22	8·98	365	4·46	8·84	9·28	9·25
1931–2 . .	363	3·27	8·99	365	4·53	8·87	9·30	9·29
1932–3 . .	120	3·38	8·78	118	4·39	8·77	9·09	9·17
Whole period .	1565	3·25	8·94	1550	4·54	8·84	9·24	9·26

It must not be assumed that there is no difference in fat content between morning and evening milk when the periods are both of twelve hours duration. Campbell[2] has shown that under winter conditions night itself encourages a greater yield of milk at the morning milking and a slightly lower fat percentage.

Similar effects are to be observed when cows are milked thrice daily. The observations of Cruickshank[3] summarised in the following table show the varying extent to which individual cows react to unequal milking intervals, not only in fat percentages, but in the weight of fat per milking.

	1 Noon milking		2 5 p.m. milking		3 5-30 a.m. milking		Difference 2-3	
	% Fat	lbs. Fat	% Fat	lbs. Fat	% Fat	lbs. Fat	% Fat	lbs. Fat
Cow 1 . .	4·70	0·59	4·35	0·44	3·25	0·68	1·10	−0·24
Cow 2 . .	4·65	0·67	5·60	0·64	2·75	0·50	2·85	+0·14
Cow 3 . .	4·25	0·83	4·70	0·68	2·30	0·62	2·40	+0·06
Cow 4 . .	5·25	0·97	4·50	0·56	2·65	0·74	1·85	−0·18
Cow 5 . .	4·75	0·88	5·20	0·76	2·20	0·55	3·00	+0·21

[1] W. L. Davies, 1939, *The Chemistry of Milk*, p. 26.
[2] Campbell, 1932, *J. Dairy Res.*, 4, 28.
[3] Cruickshank. Quoted in miscellaneous publication No. 65, Min. Agric.

When this disturbing effect of a long night interval coincides with other factors, such as a large proportion of early lactation cows in the herd or the change-over from stall feeding to grazing on spring pastures, there is a serious risk of the fat percentage of the morning milk falling below the 3 per cent fat limit. In the course of their experiments on the composition of milk from a herd of ten shorthorn cows, Golding *et al.*[1] found that on 147 occasions out of 1,074 in the first three-year period the morning milk fell below 3 per cent fat. The large majority of these instances occurred in late April and May when grazing commenced.

It is realised that many considerations are involved in fixing the hours of milking of any particular herd; but if it is at all possible to shorten the night interval at such times when low fat percentages may be anticipated in the morning's milk, the possible avoidance of legal disputes under the Sale of Foods and Drugs Act would amply compensate any inconvenience arising from a temporary readjustment of milking hours.

Efficiency of Milking.—This factor is in the main concerned with yield and fat percentage per milking. As milking proceeds, the percentage of fat increases from about 1 to 2 per cent in the first drawn or fore milk to 6 or more in the strippings. For reasons already explained, the percentage of non-fatty solids will show a decrease as the fat content increases, but when calculated on a fat-free basis there is practically no alteration. The necessity for complete milking for the maintenance of yield and fat content is at once apparent. Turner[2] has observed that when the quarters are milked separately there is a decrease in fat percentage in the milk drawn from the last quarter. This effect he attributes to a reduction of pressure within the udder. Both he and Mackintosh[3] are agreed that to ensure a satisfactory fat percentage, the milking must be done quickly and thoroughly. It is a common observation that the employment of unskilled milkers can be detrimental to both yield and quality of the milk.

From the foregoing considerations it becomes apparent that the three factors—breed, hours of milking, and efficiency of milking—exert a dominant effect on the fat content of milk.

Yield.—With increasing yields per milking the percentage of lactose increases while fat and non-fatty solids decrease. Whether the yield affects the composition or vice versa is perhaps an open

[1] Golding, *et al.*, 1932, *J. Dairy Res.*, 4, 48.
[2] Quoted by Roadhouse and Henderson, 1941, *The Market Milk Industry*, p. 16.
[3] Mackintosh, 1933, *Berkshire Farmer's Year Book.*

question. Gaines[1] considers that the percentage of fat is merely a mathematical expression of the ratio of the average rate of fat secretion to that of milk secretion. In his view the yield varies inversely as the energy value of the total solids per unit weight of milk.

Low yields may be caused by advanced lactation, disease, or conditions of drought, *but in the two latter cases low percentages of non-fatty solids are manifest.* One reason for this effect is that in order to rectify the reduction of osmotic pressure caused by the low lactose concentration, the cow increases the concentration of chlorides and soluble non-protein nitrogenous compounds, which although restoring the osmotic pressure to its normal level, are unable to effect complete rectification of the loss in percentage S.n.F. Further attention will be given to this matter in a later section.

Lactation Effects.—Bearing in mind the fundamental principle of milk secretion, namely, providing for the needs of the calf, some modification of composition is bound to occur as those needs alter during the early days of its life. Colostrum, the first secretion after parturition, is therefore very different from milk in its composition and general properties. The chief characteristics are its viscous nature, high percentage of globulin, a low lactose content, and high chlorides. On account of the high percentage of soluble protein, colostrum coagulates on heating, but this property is lost as the concentration falls to normal during the gradual change of the secretion from colostrum to milk. Garnet and Overman[2] summarise the observations of some twenty-five investigators as follows:

Composition of Colostrum

	% Total solids	% Fat	% Total protein	% Lactose	% Ash	S.G.
1st milking	24·55	3·89	16·76	2·50	1.33	1·0604
2nd ,,	18·0	3·84	9·33	3·52	0·97	1·0437
3rd ,,	16·79	3·11	7·06	3·85	0·96	1·0376
4th ,,	15·21	3·82	6·16	4·23	0·88	1·0372

Elsdon[3] has determined the freezing-point depression of colostrum and finds it to be greater than that of normal milk. As the

[1] Gaines, 1928, *Bull.* 308, Illinois University.
[2] Garnett and Overman, 1940, *J. Dairy Sci.*, 23, 13.
[3] Elsdon, 1934, *Analyst*, 59, 665.

secretion changes to normal so the freezing-point depressio moves gradually to the normal level. To meet the special need of the very young calf, the quantities of carotene, vitamins A, B and D, and iron are much greater in colostrum than in milk.

Garnett and Overman[1] estimated the major constituents an the mineral elements in colostrum from a first calf Ayrshire heife Their very comprehensive figures are shown in the table o p. 83. They illustrate the gradual change from colostrum to mil very clearly.

During early lactation the yield of milk increases to a maximum and then declines steadily to the end of the period. In view o the foregoing observations on the reciprocal relationship betwee yield and percentages of fat and non-fatty solids, it is to b expected that the latter should decline to minimum value corresponding to the peak of production. The extent of thi decline varies considerably with different individuals, but recorde observations indicate that for fat, 0·2–0·4 per cent, and for non fatty solids 0·2–0·3 per cent may be anticipated. These figure must be accepted with reservation. Fat is certainly affected mor than the non-fatty solids, and the extent of this decline is greate in morning's milk.[2] When a considerable proportion of the herd reach this minimum period simultaneously the mixed milk wil be somewhat below normal in total solid matter. If this period coincides with the commencement of grass feeding, there is a risk of the morning milk falling below the 3 per cent fat minimum particularly from herds whose general average is not far removed from this figure. At such times the inequality of milking interval will augment this effect.

Observations of the precise period at which this minimum occurs are very divergent, figures from three to sixteen weeks after calving having been quoted. Breed, individuality, condition of the cow before calving and subsequent management, are all known to modify lactation effects. Once the minimum period is passed, there is a gradual increase in fat content which becomes more marked during the last month of lactation. After the initial decline, the percentage of non-fatty solids is modified by age and pregnancy. In the first lactation it follows to a lesser degree the same trend as the fat, but in subsequent lactations it suffers a further steady decline. Barrenness aggravates the latter tendency.[3]

[1] Garnett and Overman, 1940, *J. Dairy Sci.*, 23. 13.
[2] Drakeley, 1927, *J. Agric. Sci.* 17. 118.
[3] Bartlett, 1934, *J. Dairy Res.*, 5, 113.

	%T.S.	%Fat	%Protein	% Lactose (diff.)	S.G.	%Ash	%CaO	%MgO	$\%K_2O$	$\%Na_2O$	$\%P_2O_5$	%Cl
At parturition	25·38	5·4	14·70	4·12	59·4	1·16	·288	·056	·150	·106	·439	·122
After 6 hrs. .	21·15	4·85	11·36	3·91	45·7	1·03	·216	·020	·182	·067	·282	·117
12 hrs. .	20·32	8·46	7·00	3·95	38·9	·91	·199	·031	·168	·097	·325	·121
18 hrs. .	14·84	4·90	4·44	4·67	34·2	·83	·176	·022	·183	·092	·298	—
24 hrs. .	13·70	4·43	—	—	34·3	·79	·174	·022	·185	·088	·295	·117
32 hrs. .	11·65	2·31	4·09	4·38	34·6	·87	·186	·022	·214	·070	·302	·105
40 hrs. .	13·81	3·92	4·17	4·81	34·5	·91	·202	·023	·217	·071	·343	·091
48 hrs. .	12·22	3·14	4·06	4·16	32·2	·86	·192	·023	·205	·078	·314	·100
56 hrs. .	12·52	3·23	3·73	4·74	33·2	·82	·176	·020	·179	·073	·275	·102
64 hrs. .	12·98	4·25	3·83	4·08	32·1	·82	·178	·023	·193	·070	·284	·094
72 hrs. .	12·87	4·00	3·86	4·16	32·4	·85	·183	·025	·196	·076	·286	·096
10 days .	13·56	3·73	3·17	5·91	36·1	·75	·168	·018	·183	·063	·252	·068

NOTE.—T.S. = Total solids. Figures in the S.G. column represent the lactometer readings, i.e. 1000 (S.G. − 1).

The observed differences between the maxima and minima of percentages of fat and non-fatty solids range from 0·3–1·2 for the former and 0·3–0·5 for the latter. There are very considerable differences between individuals in this respect and these figures must be accepted with reserve. Like most generalisations these statements will be found occasionally to be inapplicable to certain individuals. The author has collected records of cows which show *precisely the contrary* lactation effects, and there is no doubt that individuality strongly dominates this factor.

Lactation brings about changes in fat composition in so far as the proportion of steam volatile soluble fatty acids decreases regularly throughout the whole period. It is also of interest to find that there is a simultaneous reduction in the size of the fat globules. Of the individual constituents of the non-fatty solids, *Lactose* shows a gradual fall of about 0·5 per cent from the commencement to the end of lactation. Once the minimum period is passed, the regular fall in lactose is counterbalanced to some extent by increases in *protein*. This subject has been investigated by Azarme,[1] who observed a rapid decline in the percentage of total protein over the first four weeks of lactation followed by a gradual increase which becomes more pronounced at the end of the period. Although similar effects were noticed with all three individual proteins it is important to note that their relative proportions alter to such an extent as to give low casein numbers (per cent Casein nitrogen/per cent total nitrogen) in advanced lactation.

Ash constituents. Several workers have observed that chlorides increase as lactation advances. Sharp and Struble[2] noticed a rapid fall to the normal level very early in lactation, followed by a steady increase over the first 60 per cent of the period, while the last ten per cent showed much more rapid increases. It is a common observation that a salty taste may be detected in some samples of milk from cows in advanced lactation. Calcium appears to rise steadily after an early decline to the minimum concentration, whereas the total phosphoric tends to decline throughout. As very few complete mineral analyses have been made on milk at various stages of lactation, there is at present little evidence with regard to its effect on the remaining mineral constituents.

[1] Azarme, 1938, *J. Dairy Res.*, 9, 121.
[2] Sharp and Struble, 1935, *J. Dairy Sci.*, 18, 235.

Natural acidity commences at a high level from which it falls to a normal value during the first two or three months, after which it remains fairly steady until the last month, when it decreases sharply. The two extremes may differ by as much as 0·05–0·06 per cent calculated as lactic acid.

Season.—Only when the many other disturbing factors are eliminated is it possible to observe the effect of season on the composition of milk. From an examination of many thousands of samples of mixed milk over a number of years[1] [2] [3] it becomes evident that both fat and non-fatty solids show slight but well defined variation during the course of the year. The graph of fat percentage plotted against months of the year is in the form of a 'V', with the minimum in June and the upper extremities at January and November. The difference between the extremes is of the order of 0·3–0·4 per cent. A similar graph for the non-fatty solids takes the form of an oblique 'W' showing the highest values in January and December. The lower values occur in April and August, but between these two months there is a very slight increase. Extreme values differ by about 0·1–0·2 per cent. In the paper by Cranfield and Baker the suggestion was made that the yearly rainfall of the Midland area concerned in the investigation showed some correlation with the average yearly percentages of fat and non-fatty solids. Further evidence is needed to test the validity of this suggestion, but it is a matter of great interest, particularly in view of the observations on the effects of drought which have been made by various workers.

In an earlier section it became necessary to emphasise the limitations of averages. The matter arises again in that most practical problems are concerned with isolated herds of comparatively few cows where the other disturbing factors are by no means eliminated. In such cases it is rare that season in its meteorological sense is ever allowed to act independently. So far as milk production is concerned the term "season" embraces a multitude of separate factors, such as temperature, hours of sunshine, length of day, exercise, rainfall, drought, stall feeding or pasture feeding, influx of newly calved cows, thunderstorms, etc. It is not surprising, therefore, that observations regarding the effect of this complicated association of factors on the composition

[1] Richmond, *Dairy Chemistry*, 1930, p. 306.
[2] Baker and Cranfield, 1933, *J. Dairy Res.*, 4, 246.
[3] Elsdon and Walker, Richmond's *Dairy Chemistry*, 1942, pp. 51, 52.

of milk should so often appear contradictory. There is, however, a good measure of agreement on the following points:

(*a*) Increase of temperature favours a slight decrease in fat percentage and vice versa.

(*b*) The quantity of vitamin D in milk is directly related to the exposure of the cows to sunlight, summer milk being richer in this respect than winter milk.

(*c*) Both carotene and riboflavin reach a high level in summer milk.

(*d*) Conditions of summer drought depress the percentage of non-fatty solids, but are apparently without effect on the fat percentage.

Cranfield[1] records that in September 1929, of 49 samples of mixed milk 22 were below the minimum limit of 8·5 per cent non-fatty solids. Golding *et al.*[2] in the course of their work on the composition of the milk of a herd of ten Shorthorn cows, observed that during the drought of August 1933, both morning and evening milks were consistently below 8·5 per cent S.n.F. Whether these effects are the direct result of an abnormal fall in yield or to physiological disturbances, or indeed to both, cannot be stated with any degree of certainty. There is no evidence that similar results would be produced by drought at other periods of the year. Thus, Stubbs[3] found that a period of drought occurring in February to May 1938, was without effect on the percentages of fat and non-fatty solids.

Effects of the Commencement of Pasture Feeding.—While the popular view is that a fall in both fat and non-fatty solid percentages may be anticipated when the cows commence grazing, it is important to note that there is evidence for and against this belief. The available evidence has been presented by Elsdon and Walker,[4] who remark upon its scarcity and lack of conformity. They draw no conclusions from it. Possibly the new grass has a depressing effect on the fat percentage of the milk of spring-calved cows, whereas it may have little effect on that of the autumn calvers. Eckles, Combs, and Macy[5] observed that the latter may produce milk which does not follow the general seasonal tendency. Graphs are shown to illustrate the steady fat content of such milk during the spring and summer.

[1] Cranfield, 1930, *J. Min. Agric.*, 37, 347.
[2] Golding *et al.*, 1935, *J. Dairy Res.*, 6, 6.
[3] Stubbs, 1939, *Analyst*, 64, 672.
[4] Elsdon and Walker, Richmond's *Dairy Chemistry*, 1942, pp. 40-44.
[5] Eckles, Combs, and Macy, 1936, *Milk and Milk Products*, p. 50.

The author[1] followed the composition of the milk from the college herd over the period October 1935 to November 1936. and found a close relationship to exist between the monthly lactation composition of the herd and the observed variations in fat, S.n.F., natural acidity, etc., so close indeed that some changes usually ascribed to 'season' might well have been lactation effects.

Influence of Feeding.—Given a well-balanced, palatable ration fed strictly in accordance to milk yield, the question often arises whether the substitution of one kind of feeding stuff for another can raise the fat percentage of milk. Except in a few special cases the weight of available evidence points to a negative answer.

Some very interesting experiments have been conducted to ascertain the effect of feeding certain oils. There are records of increased fat percentages in milk as a result of feeding palm oil, butter fat, lard, and coco-nut oil, whereas cod liver oil or herring oil produce the opposite effect. It must be emphasised, however, that the results are variable not only from cow to cow, but with the same animal at different times. It is of interest to note that the depressants are oils of a very unsaturated type and that those which favour increases in fat content are those containing a higher proportion of saturated fatty acids. The specific effect of cod liver oil is largely removed by hydrogenation, which of course renders it less unsaturated by eliminating some of the double bonds of the constituent fatty acids.

As an example of the complexity of the problem may be quoted the results of an experiment on the feeding of soya bean oil.[2] It was found that by extracting the oil and feeding it separate from the meal an increased yield and a lower fat percentage were given. Feeding the unextracted cracked soya bean gave a lower yield of milk with a higher fat percentage. It is plain, therefore, that the problem is complicated by many new factors which still await elucidation.

That food fats are able to modify to a limited extent the composition of milk fat has been recognised for many years. The secretion of milk fat is so highly complicated that it would be absurd to expect a direct mathematical relationship to exist between them. The facts are that increases in the proportion of certain groups of fatty acids in the food fat will lead to similar

[1] Ling, 1937, *J. Dairy Res.*, 8, 173. [2] Williams *et al.*, 1939, *J. Dairy Sci.*, 22, 442.

but slight modifications in the composition of milk fat. This is particularly the case with the unsaturated fatty acids of the oleic type which are known to produce soft fats. The consistency of butter is therefore related in part to the nature of the food fats but Hilditch and Sleightholme[1] came to the conclusion that normally other factors such as the change from outdoor to indoor conditions, changes of temperature, grass feeding, exerted a more powerful influence on the composition of milk fat than the common variations in the fat ration of the dairy cow. Although it is widely accepted that grass feeding *or the circumstances associated with it*, bring about an increase in the unsaturation of milk fat, the reasons for this change in composition are still obscure.

Elsdon and Walker[2] refer to an experiment in which an increased fat percentage of milk followed an increase in the amount of protein in the ration. In this case the original ration was not adequately supplied with protein. From this and other experiments it would appear that increases in food protein beyond the maintenance and production requirements of the cow are not likely to affect the composition of milk.

Some very interesting experiments have been conducted by Powell[3] on the effect of the physical condition of the ration on the composition of milk. When the ration contained insufficient roughage in the long form, or when the amount was adequate but fed in a finely ground condition, milk of low fat content was the result. Powell was led to the conclusion that there is a definite correlation between the activity of the rumen and the composition of milk.

With regard to the percentage of individual constituents of the mineral matter of milk, attempts have been made to increase one or more of them by feeding the appropriate items in the form of a mineral supplement. With the exception of iodine, the results have been somewhat contradictory. It would appear that the cow is able to secrete milk of normal mineral status even although one or more mineral constituent is not adequately supplied in the ration. Under these conditions, the body reserves are drawn upon as a temporary expedient. The first outward sign of the inadequacy of the ration is a reduction of yield, but there is little or no effect on milk composition until the normal metabolic processes are deranged.

[1] Hilditch and Sleightholme, 1930, *Biochem. J.*, 24, 1098 ; 1931, ibid., 25, 507.
[2] Elsdon and Walker, Richmond's *Dairy Chemistry*, 1942, p. 48.
[3] Powell, 1938, *J. Amer. Soc. Animal Prod.*, p. 40. ; 1941, *J. Dairy Sci.*, 24. 504.

Two carefully controlled experiments on the effects of starvation[1] [2] have brought to light the following facts. Under these conditions there is a reduction of yield and percentage of lactose while the fat, protein and non-fatty solids show increased concentration. Smith[2] observed that when the lactose fell to an abnormally low percentage, some unidentified compound was secreted which partly restored the osmotic pressure. During the course of this work it was noticed that the composition of the milk fat was profoundly altered. Oleic acid increased very considerably at the expense of the lower fatty acids. On a molecular basis the increase of 19 molecules of the former was offset by a decrease of 24 of the latter. Incidentally this observation supports the view that normally the lower fatty acids are produced from oleo glycerides of the blood.

Under less drastic conditions of malnutrition similar increases in the unsaturation of milk fat were observed by Riddet *el al.*[3] These workers reduced the production ration to one half the required level. A reduction of yield was observed together with a fall in per cent non-fatty solids (0·3–0·5) which was felt mainly in the protein fraction.

Age.—The most recent extensive work on the effect of the age of the cow on the composition of milk has been carried out by Bartlett,[4] whose principal observations are summarised in the following table. As already observed (page 82), this factor may be more potent in advanced lactation.

	Number of Lactations								
	1	2	3	4	5	6	7	8	9
No. of cows	67	67	49	34	21	12	9	4	3
Relative yields .	100	116·6	133·4	143·7	151·8	151·9	156·3	141·4	111·6
Fat per cent	3·95	3·90	3·90	3·84	3·79	3·70	3·74	3·67	3·74
S.n.F. percentage on the fat-free milk . .	9·40	9·32	9·26	9·21	9·10	8·98	9·03	8·94	8·88

[1] Overman, 1927, *J. Agric. Res.*, 35, 637. [2] Smith, 1938, *J. Dairy Res.*, 9, 310.
[3] Riddet *et al.*, *N.Z.J. Sci. Tech.*, 1942, 23.
[4] Bartlett, 1934, *J. Dairy Res.*, 5, 179.

Excitement.—Both yield and composition are liable to transient fluctuation during periods of sexual excitement, storms, worrying by dogs or insects, but it is not possible to generalise on any one of these factors. Some cows are more sensitive to unusual conditions than others as many show competitors have discovered to their cost.

During the Second Great War some dairy herds were exposed to the ordeal of aerial bombardment. Rowland[1] observed that although in one particular herd under consideration there was a marked drop in yield there were no definite indications that the composition was altered. Hinks[2] recorded an instance in which bombing caused a substantial reduction in the percentage of non-fatty solids, although the fat was apparently unaffected. It is hoped that there will be no further need to study this particular factor.

The Composition of Milk as influenced by Hormones.—It is quite impossible in a book of this description to present an adequate treatment of the rôle of hormones in milk secretion. The reader is recommended to consult two admirable reviews of the subject by S. J. Folley[3] and W. E. Petersen.[4] The various ductless glands of the body secrete chemical compounds which are carried by the blood to other glands or direct to certain organs which they stimulate to activity. These compounds are named hormones (meaning "I rouse to activity"). On occasion they may be called upon to suppress rather than activate. Their secretion is not limited to the ductless glands, for they may be produced by the sex organs. Sometimes these compounds are regarded as chemical messengers operating from various glands under some measure of central control by the pituitary gland situate at the base of the brain. These messengers may be relayed by other glands or they may operate direct from the pituitary.

The development and activity of the mammary gland are so closely linked with those of the sex organs that it is only reasonable to anticipate that various conditions stimulating or depressing the one will not be without effect on the other. This surmise is amply justified by experimental evidence. This approach to the question of milk secretion represents one of the most outstanding developments of the past decade, and it is likely that the next few years will witness discoveries of great practical significance.

[1] *Analyst*, 1941, 66, 327, 328.
[2] Ibid.
[3] Folley, 1940, *Biological Rev.*, 15, 421.
[4] Petersen, 1942, *J. Dairy Sci.*, 25, 71.

In an endeavour to show the general trend of present research on the subject it is intended to present the reader with some of the results obtained.

One of the hormones of the pituitary gland, prolactin, is known to be responsible for the maintenance of lactation. When this substance is administered to cows past the peak of production it stimulates a higher level of production, and increases in fat percentages have been observed. Sometimes an increase in lactose concentration follows treatment with pituitary extracts.

The thyroid gland is also known to be essential for milk production. One of its hormones, thyroxine, an iodine-containing compound, controls the metabolic rate of the body. When the tempo of body processes is increased one effect is an increased percentage of fat in milk. It seemed likely therefore, that administration of thyroxine would produce these effects, and it was in fact demonstrated that the feeding of small doses of this substance to cows past the peak of production led to increases of yield and fat percentage. The same hormone exercises a depressing effect if administered at the peak of production, or at other times when the dose is too large. Kay[1] makes the interesting suggestion that one cause of the continued secretion of poor quality milk by individual cows may be an insufficient quantity of thyroxine secreted by the thyroid gland.

Oestrogen secreted by the ovaries and the female sex organs exercises two effects on lactation: one stimulating, the other depressing. In the former both fat and non-fatty solids are increased although the yield is somewhat diminished. The effect continues for weeks after the administration of oestrone is discontinued. The percentage of protein is also raised, but the casein number remains normal. The depressing effect is exercised by increased doses of the compound, and a practical problem is to discover conditions of dosage and methods of administration which favour the stimulating effect, as opposed to the depressant. Another problem is to determine whether the administration of synthetic or natural oestrone preparations is likely to have undesirable effects on the consumer as there is a possibility that the hormones may pass into the milk. Further, it is important to establish beyond doubt that hormone treatment has no detrimental effects upon the animal.

Synthetic di-ethyl-stilboesterol possesses properties similar to

[1] Kay, 1941, *Nature*, 147, 42, 75.

but not identical with natural oestrogen, and being much cheaper has been used in a number of these experiments. The stimulating effect has been obtained by injections of this compound, or by gentle massage of the udder with an ointment containing 1 per cent of the di-propionate of di-ethyl-stilboesterol. It is, however, more difficult to separate the two effects with the synthetic preparations than with the natural oestrogens. These experiments have also led to the conclusion that oestrogen stimulates the lactation hormones of the pituitary gland.

In conclusion it must be emphasised that the subject is still in experimental stage and must at present remain in the hands of expert animal physiologists.

The Effect of Disease on the Composition of Milk.

Mastitis.—The composition of milk secreted by a cow infected with mastitis shows the following characteristics:

(*a*) Low lactose and casein percentages.

(*b*) Increases in chloride content.

(*c*) Increases in the percentage of soluble nitrogen and a consequent reduction of the casein number.

(*d*) Some reduction in natural acidity.

These changes are made in such a manner as to maintain the normal osmotic pressure of milk. Thus it happens that milk of this description may have a normal freezing-point although the percentage of non-fatty solids is invariably low. Although the decrease in osmotic pressure due to the reduction of lactose is counterbalanced by increases of chlorides, the net result is to lower the non-fatty solid percentage. The explanation lies in the very different contributions to the osmotic pressure of equal weights of lactose and a chloride like sodium chloride. The osmotic pressure of a solution of salt is 10–11 times that of a lactose solution of equal concentration. A reduction of 1 per cent lactose would therefore be counterbalanced by an increase of approximately 0·1 per cent salt, leaving a deficit of 0·9 per cent in non-fatty solids.

Roland and Zein el Dine[1] have made a thorough examination of the effect of mastitis on the composition of milk. A summary of their conclusions will be given, but the reader is advised to consult the original papers which contain much valuable information.

[1] Rowland, 1938, *J. Dairy Res.*, 9, 47; Rowland and Zein el Dine, 1938, ibid., 9, 182; 1939, ibid., 10, 108, 267.

(*a*) From complaints received of dairy herds giving milk of low non-fatty solid content, it became evident that these occurrences were more numerous than was formerly believed.

(*b*) The low S.n.F. milks investigated fell into two groups.

A. Milk from cows with clinical mastitis.

B. Milk of normal appearance produced by apparently healthy cows.

The latter group was further subdivided into

b_1 Pathological, in which bacteriological examination established sub-clinical mastitis, and a much smaller group

b_2 Physiological, in which bacteriological examination did not indicate infection.

(*c*) Groups *A* and b_1 were characterised by the peculiarities of composition enumerated above, the per cent S.n.F. being sometimes as low as 4–5, and the casein number sometimes falling as low as 42.

(*d*) Group b_2, although low in per cent S.n.F., gave a normal casein number. As this effect could not be traced to mastitis it was attributed to some physiological factor.

Rowland concludes that sub-clinical mastitis (group b_1) may well account for many hitherto puzzling cases of low S.n.F. milk and also points out that the casein number is able to discriminate between the two sub-groups b_1 and b_2.

Foot and Shattock,[1] in a field investigation of 29 herds during the period January–March 1937, found that about 20 per cent of the cows in milk gave low S.n.F. at two consecutive milkings. Excluding those in advanced lactation, some 70 per cent of the low S.n.F. milks showed sub-clinical mastitis.

Other Diseases.—It is considered that diseases which cause a marked reduction of yield may be expected to modify the composition of milk. Direct evidence is not plentiful. Cranfield and Ling[2] record their observations of several years on milk of very low S.n.F. content from an apparently healthy cow. The mean values for the complete 1925–6 lactation were: 3·41 per cent fat, 7·70 per cent S.n.F., 2·97 per cent protein, 0·77 per cent ash, 3·98 per cent lactose, 0·18 per cent P_2O_5, and 0·145 per cent CaO.

Although chlorides were not estimated, it was found that the

[1] Foot and Shattock, 1938, *J. Dairy Res.*, 9, 166.
[2] Cranfield and Ling, 1929, *J. Agric. Sci.*, 19, 491.

soluble ash was abnormally high and it seems again that the composition was modified in much the same manner as occurs with mastitis. In March 1927, tuberculosis was diagnosed although a veterinary examination in June 1925 failed to establish any abnormality. The cow was slaughtered in July 1927 having been seriously ill since June of that year. Post-mortem examination revealed extensive tubercular lesions of the right lung. The disease was also very evident in the right hind quarter.

The reader may be impressed by the number of factors which are known to influence the composition of milk, and may imagine that when a herd gives milk of abnormal composition, the cause may be traced to one or more of these influences. Such is not always the case. The author has had occasion to examine the possible cause of a herd giving persistently low percentages of non-fatty solids with the negative result that none of the known factors could be held responsible. Other similar experiences are recorded in the literature, one of the most noteworthy being that of Lesser.[1] It may be that on occasion milk suffers from the physiological change in which substantial reductions of lactose are but slightly counterbalanced by small increases in chlorides. Even so, there is no known reason for this change nor is there any proved method of rectifying the condition. It may be the result of unbalanced hormonal control as was suggested by Kay, but *it must be admitted that at present our knowledge is far from complete.*

The Composition of Milk, Legal Considerations.—The ease with which milk may be adulterated by addition of water or separated milk without appreciable visible effect makes it imperative to combat such criminal practices by appropriate legislation and the thoroughness with which these measures are put into effect reflects the public appreciation of the high nutritive value of milk. Under the provisions of the Sale of Foods and Drugs Act, 1938, the addition to milk of any of the following substances is forbidden: water, dried milk, separated milk, condensed milk or liquid reconstituted from these two products, a mixture of cream and separated milk, colouring matter, preservatives. Reconstituted milk may not be sold as milk. Provision is made for the taking of samples by the sampling officer at the place of delivery or at any time in the course of transit.

[1] Lesser, *J. Min. Agric.*, 1932, 39, 340.

The sample is divided into three parts and the bottles sealed. One is intended for analysis, one is handed to the vendor or his representative, and the remaining one is retained by the local authority for submission to the government analyst in the event of any discrepancy between the certificates of the public analyst acting for the local authority and any independent analyst to whom the vendor has entrusted his own sample.

When a sample is known to originate from any particular herd of cows, the vendor may within 60 hours request the local authority to obtain a sample from a corresponding milking, it being understood that the cows are efficiently and fully milked and that no changes in milking intervals are made. This sample is known as the appeal sample and is sometimes taken at the request of the public analyst in order to assist his interpretation of the results obtained from the former sample.

A provision of the Act gives the Minister of Agriculture powers to establish certain minimum limits of composition of milk and separated milk which shall form the basis upon which a presumption of adulteration may be raised. These are contained in the "Sale of Milk Regulations, 1939," and are to the effect that if a sample of milk (other than separated milk or condensed milk) is found to contain less than 3 per cent of fat or 8·5 per cent non-fatty solids, it shall be presumed, until the contrary is proved, that the milk is not genuine by reason of abstraction of fat or non-fatty solids, or by the addition of water. A similar clause relating to separated milk fixes the minimum limit of composition as 8·7 per cent non-fatty solids.

So much contention has arisen over this particular section of the Act that some more detailed examination is necessary. It is apparent that the limits 3 per cent fat and 8·5 per cent non-fatty solids are well below the average for normal milk and there are some who would argue that on this account the production of low quality milk is encouraged. Others there are who contend that as genuine milk is known on occasion to fall below these limits, there may be many unjust convictions. The former argument is countered by the latter since any increases in the minima would lead to many more unjust prosecutions. The latter argument cannot be denied but any further lowering of the minima would not be in the public interest. The figures therefore represent a compromise between these two points of view.

The report of the County Analyst for the County of Lancaster for 1928 refers to the fact that less than 1 per cent of the samples examined during the previous nine years from some 25,000 herds fell below the legal limits of composition.[1] Although in this particular case the *percentage* number of *possibly* unjust prosecutions is undoubtedly small, the fact that such possibility might exist is a matter of great concern to producers, vendors, and local authorities.

It should be noted that where a sample of milk is below the minimum limits of composition, the law recognises that it *might* be genuine and therefore the figures themselves are not taken as *proof* of adulteration. *They merely raise a presumption to that effect.* But the onus of proving it to be genuine falls upon the defendant. This is a reversal of the normal procedure in which the defendant is assumed innocent until the prosecution has established proof to the contrary.

Another important matter arising from this particular clause of the Act is that there is no implication that a sample of milk is genuine merely because it happens to contain more than 3 per cent fat and 8·5 per cent non-fatty solids. It is possible for a local authority to obtain proof of adulteration and to take the appropriate proceedings in spite of the fact that the milk as sold was not below the minimum limits of composition.

To avoid unjust penalisation of innocent persons it is clear that the spirit rather than the letter of the law should be the guiding principle. Much depends upon the method of giving effect to these regulations and it is particularly gratifying to note that of recent years there has been on both sides a better understanding of the difficulties involved.

The number of cows contributing to the sample is obviously an important factor to be considered in assessing correctly the analytical results. As shown on page 74 there is a much greater possibility of small herds giving milk of low quality than is the case with larger ones.

Owing to day-to-day variations, comparison between the analyses of the sample in question and the appeal sample may be misleading and here again, the number of cows in the herd will modify the extent of this daily fluctuation in composition.

From all of the foregoing considerations it is clear that deficiencies of fat and non-fatty solids cannot be held to prove

[1] Elsdon, 1929, *Analyst*, 54, 465.

adulteration, and that *unless there is definite proof, the use of formulæ such as the following is fundamentally unsound.*

$$\% \text{ added water} = \frac{100\ (8{\cdot}5 - \text{S.n.F.})}{8{\cdot}5}$$

$$\% \text{ Fat removed} = \frac{100\ (3 - \text{F.})}{3}$$

Having proved addition of water or removal of fat, it appears to the author a matter of little significance to determine the precise extent of the offence.

An analysis by Messrs. Bibby of milk prosecutions has revealed the fact that, in their survey, one-third of the cases had arisen from an inadequate bulking of the herd milk. It is easy to see that where the milk is transferred from the milking bucket direct to the cooler, there will be inequalities in the composition of the milk as one churn after another is placed under the cooler. This may be remedied by the use of a milk 'equaliser' by which means three or five churns may be filled simultaneously, when there will be no differences in composition from churn to churn.

When milk from the milking bucket is run direct over the cooler into bottles there are likely to arise considerable differences in composition from bottle to bottle. The only satisfactory way out of this difficulty is to bulk the milk before bottling, but in some cases the practical difficulties involved would be very formidable. In the same survey it was found that many cases had arisen through failure to mix the milk before selling to the consumer. Whether through ignorance or indifference there are still retailers who draw milk from a tap at the bottom of the churn into a smaller receptacle from which the customer is served. As a consequence there may be a very uneven distribution of fat from the beginning to the completion of the round. If the bulk happens to be just above the 3 per cent fat level there are serious risks of some customers receiving milk below the legal minimum of composition.

Since it is not possible by estimations of fat or non-fatty solids to determine whether a sample is watered or whether it is genuine milk of low quality, it is a matter of great importance to know what further analytical data would serve to discriminate between these two possibilities. Several tests have been advocated from time to time, but only the freezing-point determination has received any general measure of acceptance.

The Freezing-point Test.—There is no doubt that the freezing-point of milk is its most constant property. During the past ten

years a great mass of evidence has established this fact, and the equally important one that abnormal milks of low non-fatty solid content possess normal freezing-points. If, however, the low percentage of S.n.F. is due to watering the freezing-point will be higher than normal, i.e. the freezing-point depression will be less. Actual observations indicate a slight tendency for abnormal milks to show lower freezing-points (greater freezing-point depressions) than normal. It is necessary to point out that to obtain the true freezing-point a number of corrections have to be applied to the observed reading, but when the results are required for comparative purposes only, these corrections may be omitted provided the apparatus and technique are standardised. The commonly used apparatus and technique are those of Hortvet, and it is customary to record the freezing-point as—°C. (Hortvet).

Elsdon and Stubbs[1] examined 1,000 samples by this method with the following results:

FREEZING-POINT DEPRESSION °C.

	A.M.	P.M.	Unknown	Total
No. of samples	440	500	60	1000
Minimum . .	0·530	0·529	0·530	0·529
Maximum. .	0·563	0·563	0·556	0·563
Average . .	0·545	0·544	0·544	0·544

Their survey shows very clearly that there is no correlation between the freezing-point and percentage of non-fatty solids; for example, four samples in the group containing 7·6 to 7·9 per cent S.n.F., which under the Act would be presumed adulterated, gave freezing-point depressions ranging from 0·535 to 0·555, with an average of 0·545. Five samples in the 9·5 to 9·6 per cent S.n.F. group gave freezing-point depressions 0·539 to 0·550, average 0·547. They found the freezing-point to be unaffected by seasonal changes. *In 1931 these authors came to the conclusion that the average freezing-point depression of milk was 0·54°C., but unless this value fell below 0·53°C., there was no evidence of watering. Their further experiences have fully confirmed this conclusion.* Provided the apparatus and technique are standardised the results obtained by different workers on the same sample of milk will show no appreciable differences. A sample was divided into six

[1] Elsdon and Stubbs, 1934, *Analyst*, 59, 146.

parts and the freezing-point depressions, as found by six independent analysts, were: 0·549, 0·550, 0·552, 0·549, 0·551, 0·549.[1]

Aschaffenburg and Temple[2] found no evidence that the stage of lactation has any influence on the freezing-point of milk, but a very slight but definite decrease in the freezing-point depression was noticed at the time of year when spring pastures became available to the animals. This effect was found to persist for several weeks.

Andrew[3] records an instance of the mixed milk of a herd containing 4·2 per cent fat and 8·2 per cent non-fatty solids. Although a presumption of added water would be raised on this analysis, the freezing-point as determined by a somewhat different method proved to be normal, namely, —0·55°C. Four of the cows were found to be suffering from mastitis and their milk gave the following analytical data:

	Cow 6	Cow 8	Cow 10	Cow 13
% Fat . .	4·00	2·40	4·00	3·70
% S.n.F. .	7·80	5·76	7·54	8·00
Freezing-point	—0·555°C.	—0·550°C	—0·560°C.	—0·560°C.

There are two serious limitations of the freezing-point test. It is unable to detect addition of separated milk, which form of adulteration is far more intelligent than addition of water. For the same reason it cannot detect removal of fat. Further, the freezing-point of milk is seriously affected by the development of acidity. With regard to the first limitation, the reader is reminded that the freezing-point being a function of the osmotic pressure, is determined by substances in solution, and that suspended matter of the nature of fat globules are therefore without effect on this property.

The second limitation is serious in so far as the effect of developed acidity is opposite to that of added water. By bringing more mineral matter into solution, the development of acidity increases the osmotic pressure and an acid sample will have a correspondingly greater freezing-point depression. Watered milk may therefore escape detection if it arrives at the laboratory in a very acid condition. It is true that a correction may be employed

[1] Elsdon and Stubbs, 1934, *Analyst*, 59, 585.
[2] Aschaffenburg and Temple, 1941, *J. Dairy Res.*, 12, 315.
[3] Andrew, 1929 *Analyst*, 54, 637.

whereby the freezing-point of the fresh sample is calculated from the observed value, but there is an undesirable margin of uncertainty owing to the fact that the customary titration of milk estimates lactic acid plus natural acidity, the latter being a variable quantity. Elsdon and Walker[1] suggest a deduction from the observed freezing-point depression of 0·003°C. for every 0·1 ml. above 2 ml. of N/10 NaOH used in titrating 10 ml. of milk. In terms of the more commonly used N/9 NaOH this correction would amount to 0·0033°C. for every 0·1 ml. of alkali above 1·8 ml. required per 10 ml. milk. Lockwood[2] suggests that if the sample is sufficiently acid to give a red colour when treated with 0·1 ml. of a 0·1 per cent solution of methyl red the freezing-point test should be abandoned. It is very desirable that the test should be carried out on samples containing as small a quantity of lactic acid as possible. *If, therefore, the vendor desires to use his own sealed sample as a check on the findings of the local authority, he should send it immediately to an independent analyst for estimations of freezing-point (Hortvet) fat and S.n.F. percentages.* If this third sample is kept until it curdles it will be impossible for an analyst to arrive at a true estimate of the original freezing-point and, moreover, the results of the fat and solids estimations will not be as accurate as when determined on the fresh sample.

Evans[3] has made a courageous attempt to reconstruct from the sour sample a solution which would give the freezing-point of the fresh milk, but until further observations are to hand the method must remain in the experimental stage. Other suggestions whereby this difficulty might be overcome include packing the sample in a suitable container provided with an ice jacket, or the use of a preservative which would not affect the freezing-point of the sample. Lockwood[4] finds that a solution of formalin carefully prepared so as to have the freezing-point of normal milk is quite efficient in preserving milk for several days even when used in small quantities. Macdonald[5] has described how the original freezing-point may be found from the freezing-point of preserved samples and their determined formaldehyde content.

In view of the common practice of pasteurisation, it is a matter of considerable importance to know whether the freezing-point is affected by such treatment. The matter has received very close

[1] Elsdon and Walker, 1942, Richmond's *Dairy Chemistry*, p. 110.
[2] Lockwood, 1932 *Analyst*, 57, 698.
[3] Evans, 1936 *Analyst*, 61, 666.
[4] Lockwood, 1932, *Analyst*, 57, 698.
[5] Macdonald, 1943, *Analyst*, 68, 171.

attention, as a result of which, it is found that although pasteurisation and sterilisation are not entirely without effect, they are unlikely to interfere with the normal interpretation of the results of the test.

It is not proposed to deal with practical details in this section, but the author wishes to make it clear that the determination is not one to be entrusted to an assistant who has not received thorough training in all its technicalities, and whose skill in operating the test has not been thoroughly checked from time to time.

The Nitrate Test for Added Water.—Since natural water supplies often contain nitrates, whereas milk contains no appreciable traces, it is clear that the presence of nitrates in milk may be taken as evidence of watering. The test has many limitations, for example, their absence cannot be taken as a criterion of genuineness because some public water supplies are free from nitrates. Another limitation lies in the fact that nitrates in milk are slowly reduced and, as a consequence, an acid sample which originally would have given a positive result may escape detection.

Vieth's Ratio.—This, like the nitrate test, is valuable in furnishing confirmatory evidence to the freezing-point test and other analytical data. Vieth found that the ratio of percentages lactose: protein: ash for normal milk is 13 : 9 : 2. Abnormal milk, i.e. genuine milk low in percentage S.n.F., has a different ratio owing to the lower lactose and higher chloride content. Watering a normal milk dilutes each of these constituents to the same extent, and there is thus no alteration in this ratio. If a sample of low non-fatty solid content is found to have a normal Vieth ratio a very strong presumption of added water is raised and valuable evidence is supplied to support that of the freezing-point test.

Refractive Index of Milk Serum.—By the addition of a prescribed copper sulphate solution to milk, the colloidal matter is precipitated and on filtering a clear serum is obtained which may be examined with a dipping refractometer. From the reading of the instrument, the refractive index of the serum may be calculated if desired, but in most cases the reading alone suffices. The average reading at 20°C. is approximately 38, and in the opinion of some workers a reading less than 36 would indicate added water. As the value is known to be at least as variable as the percentage S.n.F., it cannot be relied upon to detect added water, and must therefore be regarded as a confirmatory test. It suffers from the same effects of acidity as does the freezing-point test,

because the refractive index increases with increasing concentration of the serum. The most serious limitation lies in its inability to discriminate between watered milk and abnormal samples.

Regarding other forms of adulteration, it is impossible at present to detect addition of separated milk or removal of fat, so that reliance must be placed on the estimation of fat, which, if below 3 per cent, raises a *presumption* of adulteration.

CHAPTER IV

THE EFFECTS OF HEAT ON MILK AND THE ACTION OF MILK ON METALS. DAIRY DETERGENTS. CHLORINE STERILISATION

In an earlier chapter reference has been made to the changes brought about by heat on the various milk constituents, but it is convenient to regroup and supplement these observations under one heading.

The effects of heat on milk depend upon three factors—temperature, time of holding, and *p*H. The first two are very closely related, as may be seen from the following figures relating to the heat coagulation of milk:[1]

Temperature °C.	131	128	123	116	114·5
Time in minutes for coagulation to occur	10	12	20	45	60

In this particular instance the relationship is logarithmic. The third factor is of variable effect, but may be illustrated by the observations of McInerney on the effect of added lactic acid on the temperature of heat coagulation of milk:[2]

Percentage acidity	0·25	0·35	0·40	0·50	0·57
Temperature of coagulation °F.	180	150	100–110	75–80	60–65

Some of the more obvious effects of heat are a slight browning, the development of a cooked flavour, the formation of a skin, and some interference with creaming properties.

Browning and flavour development are closely related, and are much more apparent in sterilised than pasteurised milk. Although pure lactose does not caramelise at the temperatures employed in pasteurisation or sterilisation, it is known that lactose is able to form complexes with proteins and basic nitrogenous compounds which 'caramelise' at lower temperatures. Albumin and globulin also impart a cooked flavour to milk when heated. An odour reminiscent of sulphuretted hydrogen is frequently noticed when

[1] Holm, Deysher, and Evans, 1923, *J. Dairy Sci.*, 6, 556.
[2] McInerney, 1920, *J. Dairy Sci.*, 3, 220.

milk is heated, due to some measure of decomposition of soluble proteins or of compounds absorbed on the fat globules. In this connection Josephson and Doan[1] attribute the cooked flavour of milk to the formation of sulphydryl derivatives when milk is heated above 66°C. (170°F.).

The composition of the skin which forms on the surface of milk gives a valuable indication of its mode of formation. It consists of protein with variable amounts of fat, and mineral matter which approximates in composition to tri-calcium phosphate. When milk is heated there is a concentration of protein at the milk-air surface, and this, coupled with the constant evaporation of water from the surface, ultimately results in the formation of a skin. Evaporation is certainly concerned in this effect because when milk is heated in a closed vessel in which evaporation is reduced to a minimum, skin formation is very considerably retarded. An earlier view that the skin is composed of heat coagulated albumin and globulin is untenable because the skin contains a large proportion of casein. That solutions of the calcium caseinate–calcium phosphate complex exhibit this phenomenon when heated is strong evidence in support of the modern theory.

A similar process of precipitation may take place at the gas-liquid interface of bubbles or of the foam produced when milk is boiled. This ultimately leads to the formation of milk stone, which not only lowers the heat exchange efficiency of the heating coils of evaporators, but which also renders the efficient cleansing of equipment a matter of some difficulty. In the manufacture of evaporated milk, this tendency is reduced to a minimum by forewarming prior to condensing. No doubt one reason for this is the de-gassing effect of forewarming.

The effect of heat on the creaming properties of milk has been utilised by Orla Jensen[2] as a test for heated milk. Dilution of raw milk with water accelerates creaming, but with pasteurised milk it has the contrary effect. Thirty minutes holding at 145°F. (63°C.) produces an 8 per cent reduction in cream volume, whereas holding for the same length of time at 142°F. (61°C.) was without effect. The time-temperature relationship is well illustrated by the results of Marquardt and Dahlberg,[3] who determined the length of time milk may be held at various temperatures

[1] Josephson and Doan, 1940, *J. Dairy Sci.*, 23, 681.
[2] Orla Jensen, 1932, *Analyst*, 57, 383.
[3] Marquardt and Dahlberg, 1931, *N.Y. Agric. Exp. Sta. (Geneva) Tech. Bull.*, 180.

without reducing the creaming properties. Their figures are as follows:

Temperature °F. .	145	155	160
Time . . .	13 mins.	1 min.	16 secs.

There is as yet no really satisfactory explanation of this phenomenon, but Orla Jensen has suggested that heat either weakens or destroys agglutinin and as a consequence the normal clustering of fat globules does not proceed.[1] Since agglutinin is not adsorbed on the liquid globule surface it is possible that the reduction of creaming properties is in part due to the time-lag in the adjustment of the liquid globules of heated milk to the physical condition appropriate to the temperature to which it is cooled. It is well known that unless pasteurised milk is cooled to a low temperature it will show very poor creaming properties.

Bound water is also slightly affected by the heating of milk. The figures in the table are taken from the work of Pyenson and Dahle.[2]

	% Cream volume per 1% Fat	% Bound water
Raw milk unheated, aged 24 hours at 40°F.	4·23	3·75
Milk heated to 143°F. for 30 mins., aged 24 hours at 40°F. . . .	4·06	3·56
Milk heated to 160°F. for 30 mins. aged 24 hours, at 40°F. . . .	0·77	3·06
Milk heated momentarily to 180°F., aged 24 hours, at 40°F. . . .	0·76	3·30

The authors point out that it cannot be assumed that the loss of creaming properties is directly related to changes involving bound water.

In addition to the 'caramelising' effect on lactose, it has been observed that when milk is maintained near boiling-point there occurs a further decomposition of this compound. The acidity of milk which, on first heating, shows a slight decrease due to expulsion of carbon dioxide, increases at the rate of about 0·09 per cent (calculated as lactic acid) per hour when milk is kept near the boiling-point. Under these circumstances lactose decomposes into a number of acids, amongst which formic acid has been identified.[3]

[1] Orla Jensen, 1928, *Proc. World's Dairy Congress.*
[2] Pyenson and Dahle, 1938, *J. Dairy Sci.*, 21, 601.
[3] Whittier and Benton, 1927, *J. Dairy Sci.*, 10, 126.

Regarding the effects of heat on milk proteins, Rowland[1] has studied the phenomena of heat coagulation of albumin and globulin, and reiterates the viewpoint of several workers that two distinct changes are involved. Firstly, the structure of the protein molecule is altered in some obscure manner, a process known as denaturation; and, secondly, a process of flocculation or coagulation, in which the denatured particles aggregate and finally appear as a precipitate. He observed that denaturation to the extent of some 10–13 per cent of the albumin and globulin occurred when milk was held at 63°C. (145°F.) for thirty minutes. Complete denaturation was observed in samples heated to 90°C. (194°F.) for the same length of time, or as a result of the following conditions:[2] 60 minutes holding at 80°C. (176°F.), 10–15 minutes at 95°C. (203°F.), or 5–10 minutes at 100°C. It is of interest to note how closely these changes are related to losses of creaming properties[3] which occur above 60°C.

Treatment	Depth of cream layer[4] mm.	% of total albumin and globulin denatured
Raw milk . . .	12·3	0
Heated milk—		
30 mins. at 57·5°C. .	14·0	2·7
,, ,, 60°C. .	15·3	5·5
,, ,, 61·5°C. .	13·3	7·4
,, ,, 63°C. .	10·5	11·0
,, ,, 65°C. .	5·3	15·6
,, ,, 67·5°C. .	2·5	20·5
,, ,, 70°C. .	2·0	33·4
,, ,, 74·8°C. .	2·0	82·0
,, ,, 80°C. .	2·3	99·1

Rowland suggests that this process of denaturation of the soluble proteins is a major factor in the loss of creaming properties under the influence of heat. Other workers have observed that the addition of whey proteins, particularly globulin, to a heated milk would stimulate the clustering of fat globules and restore to a considerable degree the lost creaming properties.

Moir[5] has suggested that one reason for the increased time of

[1] Rowland, 1934, *J. Dairy Res.*, 5, 46. [2] Rowland, 1937, *J. Dairy Res.*, 8, 1.
[3] Ibid., 8, 195. [4] Milk allowed to cream in 100 ml. cylinders for 4 hours at 15°C.
[5] Moir, 1931, *J. Dairy Res.*, 3, 80.

coagulation of heated milk by rennet is the deposition of denatured albumin and globulin upon the casein particles. This has been neither confirmed nor disproved by other workers.

Rowland states that although no *precipitation* of denatured albumin and globulin occurs in heated milk, they are removed, together with casein, when the *p*H is brought to 4·6. It would appear that the *p*H influences precipitation rather than denaturation by heat. It is well known that when whey or casein-free milk filtrates are heated the denatured proteins appear as a precipitate and it would therefore appear that in heated milk this precipitation does not occur owing to the protective action of casein.

Heat coagulation of casein follows the same course of events, namely, denaturation and precipitation, but most observations are made on the complete change rather than the two separate stages. As this is a matter of great importance in the final sterilisation of evaporated milk, it has been the subject of many researches which have shown that the following factors are involved: temperature and time of holding, concentration of casein, presence of lactic acid, salt balance, and previous heat treatment of the sample.

The influence of temperature, time of holding, and the presence of lactic acid are illustrated by the tables on page 103. Dilution of milk increases the stability of casein to heat while concentration produces the contrary effect. In evaporated milk the temperature of heat coagulation of casein may be 20°C. to 30°C. lower than that of the original milk.

If the normal balance Ca+Mg/phosphate+citrate is disturbed in either direction the heat stability of casein is generally lowered. By forewarming milk to 95°C. it is rendered more stable to heat, and while the precise reasons are as yet unknown, it is reasonable to believe that denaturation of the soluble proteins is one contributory factor, and that a favourable shift in salt balance might be another.

As casein may be coagulated by rennet, it is not surprising to find that heated milk differs from raw milk in its behaviour towards this clotting enzyme. Heating milk for thirty minutes above 105°F. prolongs the time of coagulation. Such heated samples take some time to reach equilibrium. Further increases have been observed in the time of rennet coagulation up to five hours after heating. Denaturation of the milk proteins and

alterations in the soluble mineral constituents are certainly involved in this effect.

Heating milk for thirty minutes at temperatures above 145°F causes a decrease in concentration of soluble calcium and phosphorus, and if the treatment is severe enough, calcium phosphate and citrate are precipitated. It appears that the normal equilibrium Soluble Ca + P ⇌ Colloidal Ca + P is shifted to the right by moderate heating, beyond which the colloidal phase becomes overloaded and some precipitation occurs. There is a serious lack of agreement regarding the precise extent of these effects, but so far as calcium is concerned it may be taken that a 20 per cent reduction in the concentration of soluble calcium occurs when milk is kept at 65°C. (149°F.) for thirty minutes, and that a 40 per cent reduction occurs when milk is kept at boiling-point for one hour. Considerable losses of iodine also occur when milk is heated.

The enzymes of milk, lipase, peroxidase, amylase, reductase, catalase, and phosphatase are all sensitive to heat, and it is reasonable to suppose that their inactivation would enable the severity of heating to be judged with a fair measure of confidence. The most valuable enzyme for this purpose is phosphatase which is inactivated by comparatively low temperatures. Kay and Graham[1] found the following conditions of inactivation:

Heating for 20 mins. at 62·5°C. (144·5°F.)
,, ,, 5 mins. at 67°C. (152·6°F.)
,, ,, 3 mins. at 70°C. (158°F.)
Instantaneous heating to 75–80°C. (167–176°F.).

The inactivation of this enzyme forms the basis of the phosphatase test whereby it is possible to check the efficiency of pasteurisation or the admixture of raw milk with pasteurised milk.

Contrary to earlier views the losses of vitamins caused by heating milk are not very serious when milk is supplemented by other foods of the common human diet. If milk is regarded as a source of vitamins A and B_2, the losses caused by pasteurisation, or even sterilisation, are very slight (page 55).

In view of the increasing use of pasteurisation it is a matter of great concern to know whether this practice is detrimental to the nutritive properties of milk. Very many experiments have been conducted in both human and animal nutrition, some showing a slight superiority of raw over pasteurised and others being inconclusive or even indicating the contrary effect. Of these

[1] Kay and Graham, 1933, *J. Dairy Res.*, 5, 63.

experiments that known as the Lanarkshire experiment is noteworthy. The growth response of school-children of both sexes between the ages of five and twelve was carefully recorded for groups receiving daily supplements of three-quarters of a pint of milk. The experiment involved 20,000 children living in densely populated areas. All children receiving milk supplements showed marked improvement, but the authors further stated: "In so far as the conditions of this investigation are concerned, the effects of raw and pasteurised milk on growth in height and weight are so far as we can judge equal." This experiment has been the subject of much criticism. The same figures were taken by Fisher and Bartlett,[1] who, after regrouping them, concluded that the experiment showed a definite superiority of raw over pasteurised milk. The author has no wish to reopen the controversy, but rather to state that this experiment and the various contradictory conclusions drawn therefrom illustrate the present situation very clearly. Not only do different experiments lead to different conclusions, but sometimes the results of any one experiment may be interpreted in either direction. It is impossible from the evidence at present available to state whether or not pasteurisation is likely to affect the nutritive value of milk. The author's own opinion is that so far as the normal human diet is concerned the nutritive value of milk is not significantly lowered by pasteurisation.

Milk and Metals.—Modern methods of storage, distribution, or processing result in the contact of milk with metallic surfaces for varying lengths of time, and it is therefore a matter of great importance to ascertain whether milk has any deleterious effect on the metal and vice versa. Further, as these metallic surfaces must be regularly and thoroughly cleaned and sterilised, it is equally important to know whether the metal is able to resist the wear and tear of abrasion and the attack of detergents used in these operations. It must be realised also that in the selection of a suitable metal for any particular part of dairy equipment, considerations such as mechanical strength, specific heat, and conductivity may take precedence over all others.

The Action of Milk on Metals.—Metals in contact with solutions of metallic salts will undergo a certain amount of corrosion depending on the solubility of the metal in the particular solution. Normally a point of saturation is reached beyond which no more metal dissolves, but in the presence of proteins this equilibrium

[1] Fisher and Bartlett, 1931, *Nature*, April 18.

is disturbed owing to their combination with the dissolved metal to form colloidal proteinates. Under these conditions more metal will pass into solution. The corrosive properties of milk are due not only to the various mineral salts in solution, but also to its protein content. Even in cases of extreme corrosion the actual quantity of metal dissolved by milk is very small, but the attack is renewed daily, and as a corroded surface is often difficult to clean and sterilise, further losses by abrasion and detergent action are inevitable. It will be appreciated, therefore, that the cumulative effects of corrosion may be very extensive.

Corrosion is always more serious when milk is in contact with the junction of two different metals. This is due to the creation of a potential difference between them and the consequent corrosion of whichever metal forms the anode. This type of corrosion is termed 'galvanic.' It is clear, therefore, that even where milk is without action on the metal of the particular equipment, unless care is exercised in the selection of the correct metals for solders or joints, galvanic corrosion may occur.

Aeration in general favours corrosion, and in the pioneer experiments of Hunziker, Cordes, and Nissen,[1] in which strips of various metals were immersed in milk, it was frequently observed that corrosion was greatest at the milk-air junction. Acid milk would be expected to show greater corrosive properties, but in fact this may not always be so, and one reason may be the depletion of dissolved oxygen during the development of acidity.

The conditions governing the corrosion of copper by milk are illustrated by the following figures taken from the work of Rice and Miscall.[2]

Conditions under which copper was exposed to milk	m.g. Copper dissolved per litre of milk
2½ hours at room temperature	6·00
,, ,, 145°F.	14·15
,, ,, boiling-point	4·45
1 hour at 140°F. bright surface.	5·45
,, ,, dull oxidised surface	19·90
,, ,, milk occasionally shaken	5·45
,, ,, air bubbled through the milk, occasional shaking	25·15

[1] Hunziker, Cordes, and Nissen, 1929, *J. Dairy Sci.*, 12, 140, 252.
[2] Rice and Miscall, 1923, *J. Dairy Sci.*, 6, 261.

Of the common single metals which might be used in dairy equipment, aluminium is the only one not appreciably attacked by milk. Copper and iron are not resistant, while nickel may be used only for storage or conveyance of milk at ordinary temperatures as it is attacked by milk above 18°C. Of the common plated metals galvanised iron is appreciably corroded by milk, whereas tin-plated copper and steel resist corrosion according to the efficiency of the plating. Of the two, tin-plated copper is generally superior, but in either case when the plating is worn away, galvanic corrosion may be very severe. Hunziker[1] considers that the tin-plating of surfaces which are to be heated is uneconomical owing to the frequency with which the metal requires replating.

Of the various alloys which would lend themselves to fabrication into dairy equipment, mention will be made of aluminium alloys, stainless steels, chromium nickel alloys, and alloys containing copper. Aluminium alloys containing manganese and other metals to improve the physical properties of the pure metal have proved valuable for dairy equipment, and some interesting experiments have been carried out with a view to improving their resistance to corrosion by various surface treatments. Electrolytic oxidation in an oxalic acid bath gave very promising results.[2] The composition of stainless steels varies and experience has shown the so-called 18 : 8 (i.e. 18 per cent Cr, 8 per cent Ni, 74 per cent Fe) to be most resistant. Hunziker places this alloy at the head of the list of metals suitable for dairy equipment. Alloys of this type are most attractive as they do not tarnish on exposure to the atmosphere. Chromium nickel alloys, containing 12 to 15 per cent chromium, have also proved very resistant to corrosion and are more easily fabricated than the stainless steels. Of the copper-containing alloys, nickel silver and white alloys (nickel-copper alloys) have been found unsuitable as they suffer corrosion through the copper passing into the milk, apparently unimpeded by the nickel. Henderson and Roadhouse[3] have found that the inclusion of tin and zinc in copper nickel alloy renders the metal much more resistant. The composition of the alloy was 66 per cent Cu, 20 per cent Ni, 3 per cent Sn, 4 per cent Pb, 4 per cent Zn, 2·5 per cent Fe, and 0·5 per cent Mn. It possesses good mechanical properties, and it is probable that more use will be made of alloys of this type.

[1] Hunziker, 1935, *Condensed Milk*, p. 27.
[2] Reif and Steinbeck, 1939, *Abs. Analyst.*, 64, 125.
[3] Henderson and Roadhouse, 1940, *J. Dairy Sci.*, 23, 215.

Stainless steels and nickel chromium alloys are costly, and it is interesting to find that resistant metals like aluminium or alloys like the two former may be used as a veneer over the cheaper steels. Naturally everything would depend on the efficiency of the lining process. Metals may also be lined with a 'glass' preparation. The metal is sprayed with a silica mixture which on heating, fuses into a continuous glassy lining. The vessel is then suitable for the storage or conveyance of milk, as it possesses all the resistant properties of glass. Hunziker[1] also reports that promising results have been obtained with steel tanks or vacuum pans lined with a hard rubber compound.

The Effect of Metals on Milk.—The passage into milk of any particular metal may have two undesirable consequences in addition to the foregoing observations on corrosion. It may impart a bitter, metallic flavour, and it may initiate the cycle of changes leading to an oxidised taint. With regard to the former possibility, it should be noted that in comparing one metal with another, although both may enter milk to the same extent their effect on milk flavour may be very different. Copper, zinc, and iron produce distinct metallic flavours, whereas tin, aluminium, and nickel in equal concentration may give no perceptible taint. It does not follow, therefore, that the absence of a metallic taint indicates that no corrosion has taken place. The fact that copper and to a lesser degree iron not only give a metallic flavour, but stimulate oxidative changes has led some workers to the view that they produce in milk traces of hydrogen peroxide, which is known to possess a very pronounced metallic flavour. Roadhouse and Henderson,[2] in their studies of the copper alloys, found that the disappearance of ascorbic acid from milk was a more reliable indication of the quantity of copper dissolved than was the loss in weight of the metal.

Before leaving the particular case of copper, mention must be made of the fact that in spite of the ease with which copper passes into milk, this metal is sometimes used for cheese vats and for heating coils in vacuum evaporators. In both instances solution of copper may take place, but in the former the low oxygen content of cheese does not favour fat oxidation. The vats are kept well polished, thereby reducing the quantity of copper which would be dissolved if the surface were dull. In making evaporated

[1] Hunziker, 1935, *Condensed Milk*, p. 30.
[2] Henderson and Roadhouse, 1940, *J. Dairy Sci.*, 23, 215.

milk it is customary to forewarm the milk before it passes into the vacuum evaporator. Dissolved oxygen is thereby reduced and the evaporation under reduced pressure will cause further losses. The possible anti-oxidant properties of sulphydryl compounds which may be produced has been discussed in a previous chapter (page 19). Nevertheless, Hunziker[1] observes that condenseries which have changed from copper to stainless steel are convinced that the flavour of their products is improved.

In assessing the relative merits of various metals for dairy equipment, it must be realised that solution by milk of copper, zinc, and iron is likely to prove far more deleterious than that of tin, aluminium, nickel, or chromium. Combining all the foregoing considerations, it is possible to place the common metals in descending order of their general suitability:

1. Stainless steels particularly of the type 18 per cent Cr, 8 per cent Ni, 74 per cent Fe.
2. Nickel chromium alloys.
3. Heavily tinned copper.
4. Aluminium and some aluminium alloys.
5. Heavily tinned steel.
6. Alloys of copper and nickel, containing zinc and tin (limited use until more is known of their characteristics).

Metals which must be considered unsuitable for general purposes are: galvanised iron, nickel silver, iron, zinc, copper, and most copper alloys.

The possibility of metallic contamination arising from the metal foil used for capping bottled milk must not be overlooked. Aluminium is most favoured for the purpose, although lacquered zinc foil has proved a useful substitute. When war conditions made it necessary to seek elsewhere for a suitable metal, tinned lead, tinned copper, and tin zinc alloy were examined. Kerr[2] found that under normal conditions in which the foil is in contact with the cream layer, the tinned lead allowed quantities of lead to pass into milk to a degree which would render it unsafe for human consumption. Both tinned foil and the tin zinc alloy proved satisfactory.

Dairy Detergents.—The cleansing of dairy equipment is not only a matter of removal of milk residues, etc., or the production

[1] Hunziker, 1935, *Condensed Milk*, p. 31.
[2] Kerr, 1942, *J. Soc. Chem. Ind.*, 61, 128.

of an attractive appearance to bottles and dairy plant: in addition to these very desirable aims the cleansing solution must either possess efficient sterilising qualities or it must prepare the surfaces for final sterilisation by steam. Although it may remove visible residues, water, even hot water, is unable to remove films of fat and protein which adhere to the surface of milk vessels. Recourse must be made to some more active agent.

As no one substance is effective, detergents are mixtures, the composition of which varies according to the various purposes for which they are intended. The necessary requirements of detergents intended for cleansing and sterilising milk bottles are obviously different from those of one designed for cleaning the surface of a metal tank prior to steam sterilisation. Again, a mixture designed for hand-cleansing must be without serious effect on the skin.

Sodium hydroxide, trisodium phosphate, sodium carbonate (in the form of soda ash), and sodium meta silicate are the main constituents of detergent mixtures. Other substances, such as sodium hexa-meta-phosphate, sodium sulphite, or sodium perborate, may be present in small quantities for special purposes.

It will be noticed that all the main constituents possess an alkaline reaction. This is necessary for dissolving casein and for emulsification and solution of fat. It is also necessary for the maintenance of germicidal properties which are known to depend on a correct concentration of hydroxyl ions. While all of the main constituents furnish hydroxyl ions when dissolved in water, when solutions of equal concentration are compared sodium hydroxide is pre-eminent in providing the greatest concentration of these effective ions. This substance dissolved in water undergoes strong ionisation according to the equation:

$$NaOH \rightleftharpoons \overset{+}{Na} + \overset{-}{OH}$$

The other constituents being salts of weak acids with a strong base are able to furnish hydroxyl ions by secondary reactions.

As is seen from the table,[1] showing the killing time in minutes of Staphylococcus aureus effected by various concentrations of detergent constituents at 30°C, sodium hydroxide is pre-eminent in its germicidal properties, and is therefore a desirable constituent of all detergents designed to clean and sterilise. It also acts as a lubricant in bottle-washing machines.

[1] Bottle washing, *Imperial Chemical Industries*, p. 22.

	Concentration of Solution			
	0·5%	1·5%	2·0%	3%
Sodium hydroxide	20	10	4–8	less than 2
Sodium carbonate	more than 60	60	more than 24	more than 24
Tri-sodium phosphate crystals .	,, ,,	45	,, ,,	,, ,,
Borax crystals .	,, ,,	more than 60	,, ,,	,, ,,
Sodium sequicarbonate, Na_2CO_3 $NaHCO_3$ $2H_2O$	,, ,,	,, ,,	,, ,,	,, ,,

It is considered that a *p*H of 12 is necessary for good cleansing and germicidal properties. Sodium hydroxide has a number of drawbacks. Its solutions are caustic to the skin, and for this reason, if included in hand-washing solutions its concentration must not exceed 0·25 per cent NaOH and the temperature kept below 100°F. Another serious drawback lies in the fact that it is difficult to rinse away from the surface and tends to leave glass somewhat clouded in appearance. Its adherence to a metallic surface may have a detrimental effect on the metal.

Sodium carbonate in the form of soda ash is much cheaper than sodium hydroxide, and as it is able to add its quota to the total alkalinity it is a common constituent of detergent mixtures.

Sodium meta-silicate, Na_2SiO_3, is valuable in conferring greater wetting powers to the solution, thereby increasing its penetration and expediting the cleansing action. It has good emulsifying powers and is able to minimise the risk of jet blockage in machine washing through its dispersive effect on solid matter. Because of these obvious advantages sodium silicate is a very common constituent.

Possessing excellent rinsing properties, tri-sodium phosphate is a most popular ingredient of detergent mixtures. It has very good emulsifying powers. Both the phosphate and meta-silicate possess buffering properties and no doubt serve to stabilise the *p*H of the solution.

As the composition of detergent mixtures varies considerably it is very desirable that they should be sold on a guaranteed analysis, and that the purpose for which they are intended should be stated.

A matter over which some confusion exists is the question of the effect of a hard water supply on the effective action of a

detergent solution. It is true that chemical reactions occur which involve the alkali of the detergent and the hard water, e.g., for a temporarily hard water:

$$Na_2CO_3 + Ca(HCO_3)_2 = CaCO_3 + 2NaHCO_3$$

for a permanently hard water:

$$Na_2CO_3 + CaSO_4 = CaCO_3 + Na_2SO_4$$

but the loss of alkalinity of the detergent on this account is small. A matter of much greater importance is the condition of the precipitated calcium carbonate. Normally this precipitate forms a crystalline scale on exposed surfaces which is not only difficult to remove, but which in the case of a bottle-washing machine causes loss of mechanical efficiency. In the presence of sodium phosphate and silicate, the precipitate is of a different texture, being more flocculent and less adherent to surfaces with which it comes in contact. Even so, these two substances do not entirely remove the objectionable effects of hard waters. Sodium hexametaphosphate ($NaPO_3$) prevents the formation of a precipitate when a hard water is made alkaline by the detergent and its inclusion in small quantities in detergent mixtures has proved effective in the avoidance of scale formation, etc. This compound in the form of 'calgon' is commonly used in proprietary mixtures designed for use with hard waters. It possesses two further advantages. It is free-rinsing and is effective in the removal of that type of milk stone which contains calcium caseinate and calcium phosphate. The normal detergent renders the calcium insoluble and sometimes this insoluble matter protects the casein from the solvent action of the alkali. In the presence of sodium hexametaphosphate, this precipitation does not occur and the stone is less refractory.

In soaking tanks concentrations up to 2 per cent of detergent are used. With some types of bottle-washing machines the bottles receive a preliminary soaking in 0·2 per cent NaOH, at 110°F., followed by the main soaking in 1–2 per cent detergent solution at 140–150°F. They are rinsed with a solution of sodium phosphate and sodium hexametaphosphate.[1] Higher temperatures may be used in churn-washing. In the normal working of washing machines there is a tendency for the solution to become diluted by continued entry of rinse-water at one end and the removal of small amounts of detergent at the exit. In some types of

[1] Shaw, 1943, *Dairy Ind.*, 8, 19.

machine this loss is automatically rectified by constant dripping of a concentrated detergent solution into the soaking tanks. Some measure of control may be exercised by observations of the titration values of the freshly made solution and of samples taken at definite periods of time during the working of the machine. It is possible in this manner to form some estimate of the rate of loss of strength, and if the tank capacity is known the solution may be made up to the correct strength by adding the calculated quantity of detergent. Suppose, for instance, that 10 ml. of the fresh detergent solution required 40 ml. of N/10 acid, and that after running the machine for two hours the titration value had fallen to 25 ml. N/10 acid. The loss of strength was $\frac{40-25}{40} = \frac{3}{8}$. If x lb. of the detergent had been used to make up the original solution, then provided the total volume of solution is not materially altered the addition of $\frac{3}{8}x$ lb. of detergent would rectify the loss of strength.

Regarding the effects of the detergent on metals used in dairy equipment, it must be remembered that abrasion may cause more extensive damage than corrosion by the solution. However, two examples of corrosion by the solution must be considered. As tin and tinned surfaces are soluble in alkaline detergents, it follows that if this solvent action is coupled with constant abrasion, serious damage may be caused, especially where the plating is not of highest quality. Kerr[1] has shown that the extent of corrosion of tin surfaces by the detergent is dependent on the quantity of dissolved oxygen in the solution. By addition of sodium sulphite this is effectively removed and corrosion very considerably reduced thereby. Detergent mixtures intended for use with tinned ware should contain sodium sulphite, the recommended proportion being one part of the sulphite to four of soda ash or sodium hydroxide. Aluminium is also soluble in alkalis, but it has been found that the inclusion of sodium phosphate and silicate reduces this corrosion to a minimum. Little[2] has examined the protective action of sodium perborate in this connection. He found that mixtures of sodium silicate and sodium perborate $NaBO_3$ rendered solutions of sodium carbonate and sodium phosphate non-corrosive to tin and aluminium.

The development of non-alkaline detergents is a subject of

[1] Kerr, 1935, *J. Soc. Chem. Ind.*, 54, 217.
[2] Little, 1938, *J. Dairy Sci.*, 21, 154, Abs.

great interest. It is likely that several different types will appear on the market as soon as conditions permit. One type[1] is derived from cracked petroleum. Olefines of the $C_{10} - C_{18}$ range are sulphonated and the product neutralised under controlled conditions. It behaves as the salt of a strong base with a strong acid and is not hydrolysed in solution. Being unaffected by hard water or salt solutions and being stable over a wide range of *p*H, this detergent is indeed attractive. It is not yet possible to state whether compounds of this nature are likely to be used as dairy detergents, but in the meantime their development will be followed with keenest interest.

Chlorine Sterilisation.—At the time of writing the use of chemical sterilisers for dairy plant is illegal, but the notes which follow are written in the belief that war conditions may render the legitimisation of this practice a matter of necessity.

In the previous section it was emphasised that cleaning was a necessary preliminary to sterilisation. When the detergent itself is not the sterilising agent steam is the most effective. The former is entirely dependent on the maintenance of the correct hydroxyl ion concentration, and while the efficiency of the latter is indisputable, there are farms which cannot raise the necessary steam, and even in large dairies war conditions may cause a temporary breakdown of power and steam supply. Some types of equipment such as open coolers, etc., do not lend themselves to efficient steam sterilisation. Circumstances may therefore give rise to the question, "Once cleansing has been satisfactorily carried out, is there any other means of sterilisation which can be employed with safety?"

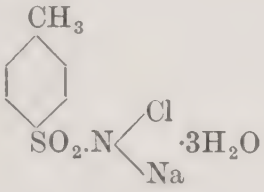
There are many chemical compounds which effectively destroy bacteria, but the majority are poisonous and cannot on that account be used for dairy utensils. The choice is strictly limited, and at present chlorine preparations are the only possibilities, since they are effective sterilisers and in dilute solutions are not poisonous. *It cannot be too strongly emphasised that these liquids possess no penetrating power, so that unless the surface is clean they cannot sterilise completely.*

The most convenient chlorinators for general purposes are sodium hypochlorite (NaOCl basis) and chloramine T. The former may be procured in the form of a solution containing 10 per cent available chlorine, which is kept as the stock solution to be diluted

[1] 1942, *Chem. and Ind.*, 62, 112.

as required. A crystalline 'hypochlorite' is obtained by the ingenious method of carrying the chlorine in the water of crystallisation of tri-sodium phosphate. This is a proprietary substance for which a patent has been granted. When dissolved in water it behaves as a buffered hypochlorite solution.

Chloramine T is a by-product of the manufacture of saccharin and has the formula



It is crystalline and forms much more stable solutions than the hypochlorites, since it gives up its available chlorine much more slowly. Because hypochlorites are therefore the more rapid sterilisers they are at present the more favoured. It was felt desirable to mention chloramine T which, in spite of its slower action, has the great advantage of being a crystalline solid which can be kept well and whose solutions are more stable. They have already proved valuable for overnight soaking of milking machine parts.

The germicidal properties of solutions of hypochlorites and chloramine T depend entirely on the concentration of available chlorine, and it thus becomes a matter of supreme importance to minimise as far as possible any loss of strength of stock solutions or of the dilute solutions as used on the equipment.

Loss of strength may arise from a variety of causes. Oxidisable matter like milk residues will consume much of the available chlorine, especially at higher temperatures, and will thus impair the germicidal activity of the solution. This again emphasises the paramount necessity for efficient cleansing before chlorination. Exposure to strong light or warm conditions of storage will also cause some loss of strength. Under favourable conditions of storage, hypochlorite solutions of about 10 per cent available chlorine should not lose more than 0·5 per cent available chlorine per month.[1]

Mattick *et al.* emphasise the need for agitation of the chlorine solution in order that the surface to be sterilised shall be in contact with constantly renewed liquid. They also stress the importance

[1] Mattick, Hoy, and Neave, 1943, *Milk Industries*, 23, 39.

of the duration of contact necessary for complete action. These two essentials they consider will be secured by using the chlorine solution in conjunction with a detergent wash. A 0·25 per cent solution of soda ash containing 300 parts per million of available chlorine is recommended, the solution to be used hot with suitable agitation by scrubbing or other means. It is highly probable that combining the hypochlorite solution with one of the sulphonated olefine detergents will prove eminently satisfactory on account of the great wetting and penetrating power of this type of compound.

The effect of metals on these chlorinating solutions and the possibility of metallic corrosion arising from their use have been the subject of a comprehensive paper by Haller,[1] Grant, and Babcock. Their experiments were conducted on two strengths of solution, 100 and 200 parts available chlorine per million, made up from sodium hypochlorite, sodium hypochlorite buffered with sodium phosphate, and chloramine T. Fifteen common metals were exposed under various conditions to the chlorine solutions, and observations made concerning the loss of strength of the solutions and the extent of metallic corrosion. Some of their results are summarised as follows:

(*a*) In contact with metals at 25°C. (77°F.), the solutions lost strength to varying degrees dependent on the extent of corrosion of the particular metals.

(*b*) All the solutions of 200 p.p.m. available chlorine were found to corrode the metals under observation. The weaker solutions were generally less corrosive.

(*c*) Hypochlorites corroded aluminium and its alloys severely, and copper and its alloys less so. Tin was untouched by the weaker solution. Stainless steels of the 18/8 type were not greatly affected.

(*d*) Chloramine T solutions attacked copper and its alloys more than other metals. Tin was untouched by the weaker solution.

Their observations on other matters concerning these solutions were:

(*e*) The presence of milk caused rapid deterioration for the first two minutes' contact, chloramine T suffering least damage.

(*f*) Under all conditions of storage chloramine T solutions were the most stable, but when kept cold and dark all were

[1] Haller, Grant, and Babcock, 1941, *U.S. Dept Agr. Washington D.C. Tech. Bull.*, 756.

equally satisfactory. At room temperature in closed vessels exposed to artificial or diffused daylight, the hypochlorite solutions were affected more than chloramine T.

If chlorine sterilisation is to become more common there will be an urgent necessity to warn dairymen that no trace of the steriliser must be allowed to pass into the milk. Under the Foods and Drugs Act this would constitute a serious offence. The detection in milk of traces of hypochlorite solutions is rendered possible by the fact that in those cases where the product does not normally contain chlorates, the manufacturers have agreed to include traces, which may be detected in milk by the method of Wright and Anderson, *Analyst*, 1938, 63, 252.

The strength of a chlorinating solution may be determined by addition of potassium iodide and acetic acid to a measured volume of the solution. A quantity of iodine equivalent to the available chlorine is liberated and may be titrated in the customary way with standard sodium thiosulphate solution.

CHAPTER V

CREAM, BUTTER, AND MARGARINE

When milk is allowed to stand for some time, an upward motion of the fat globules takes place, leading ultimately to the formation of a surface layer of milk in which the percentage of fat is considerably increased. To this fat-enriched layer is given the name cream. It will be anticipated from its mode of formation that all the constituents of milk will be present in cream, but that the fat percentage will be much higher and the non-fatty solids correspondingly lower. Cream formed in this manner will show wide variations in composition, and even when a separator is used the product will vary according to the conditions of separation.

The composition of two samples of cream is given by Richmond.[1]

	Thick cream	Thin cream
% Water . . .	39·37	63·94
% Fat . . .	56·09	29·29
% Lactose . . .	2·29	3·47
% Protein . . .	1·57	2·76
% Ash . . .	0·38	0·54

It will be seen from these figures that as the fat in cream increases, the percentages of the other constituents show corresponding decreases. In both cases the relative proportions of the non-fatty constituents are the same as for milk and the ratio water/non-fatty solids is also approximately that of milk. This enables an approximate estimate of the non-fatty solids to be made. Taking the ratio water : non-fatty solids of milk as approximately 10 : 1, if the fat content of cream is F, then the approximate percentage $\text{S.n.F.} = \dfrac{(100 - F)}{11}$.

As the fat percentage increases the cream usually becomes more viscous and quite naturally the thickness of cream has become a popular criterion of its richness. The use of thickening agents, such as gelatin, starch paste, saccharate of lime, etc., is forbidden

[1] Richmond, *Dairy Chemistry*, 1930, p. 420.

under the Sale of Foods and Drugs Act. Preservatives are also prohibited.

Conditions affecting the Rising of Cream.—Fat globules being of lower density than the liquid in which they are suspended will tend to rise to the surface, just as a stone dropped into water will sink to the bottom on account of its greater density. The same force of gravity operates in both cases, but the ascent of fat globules is opposed by a number of factors which do not possess the same significance in the parallel example of the stone falling through water. Friction on the surface of the globule will retard its upward motion, and this opposing force depends upon two factors, the viscosity of the liquid through which it moves and the surface area of the globule. The thicker (more viscous) liquids will cause a much greater retarding action than the more mobile ones. Because small globules possess a much larger surface area per unit weight than larger ones, the former will rise much more slowly than the latter. These observations are collected together in the form of a well-established mechanical formula known as Stokes' equation

$$V = \frac{2r^2(d_s - d_f)g}{9n},$$

where V = rate of ascent of the globule.
r = radius of the globule.
d_s = density of the fat-free milk.
d_f = density of the globule.
g = force of gravity.
n = viscosity of the fat-free milk.

If, from a knowledge of the size distribution of the fat globules of milk, together with the physical constants involved in the formula, the value of V is calculated for normal milk, the result will not agree with the observed rate of creaming. The discrepancy is too serious to be overlooked, for where practical observations show that creaming normally occurs in one or two hours, the calculation would indicate that it is a matter of days rather than hours. The explanation was discovered by Troy and Sharp,[1] who found that normally the fat globules form clusters of many hundreds of individuals and that owing to the enormous increase in size they rise with a much greater velocity than do the separate globules. They found that these clusters rise in accordance with Stokes' law, and observe that homogenised milk

[1] Troy and Sharp, 1928, *J. Dairy Sci.*, 11, 189.

does not cream in the normal manner because it is unable to form these large aggregates. Within the clusters, which may be spherical or irregular in form, the globules retain their individuality and a certain amount of plasma is necessarily included. The clusters contain approximately 50 per cent of fat and on reaching the surface they are packed together again with plasma between them. The depth of cream layer will depend upon the shape and size of the clusters; those of smaller size or more regular shape may be packed into a smaller cream volume than larger or irregular shaped ones. It must be pointed out that for these reasons the fat percentage of the smaller cream volume will be higher than that of the larger. Low temperatures (7–8°C. or 45°F.) are most favourable to the formation of large firm clusters, while at higher temperatures they tend to be more compact but less rigid; so that the former conditions favour deeper cream lines than the latter.

The Effect of Homogenisation.—Homogenisation of fat-enriched milk or cream has been found, under certain conditions, to result in a marked clustering of the fat globules. The facts brought out by the work of F. J. Doan[1] may be summarised as follows:

1. The homogenisation of milk or cream at 38°C. (100°F.), under a pressure of 3,500 lb. per square inch, results in a sub-division of globules from ten to one hundredfold.
2. If samples under 10 per cent of fat are homogenised, there is very little tendency for these small globules to cluster.
3. If samples over 10 per cent of fat are homogenised, there is a strong tendency for the globules to cluster.
4. Although in case 3, no creaming will occur, if the homogenised cream is diluted with milk or separated milk to a fat content of about 4 per cent, not only does creaming take place, but the cream layer is much deeper than usual. The increased volume of the cream is due to the very irregular packing of these clumps, whereby much plasma is included.
5. As the ratio S.n.F./fat of the cream increases, so the clumping tendency of the homogenised product is reduced.

Doan advances the theory that since in fresh milk there is a tendency for the fat globules to form clusters, there must be a force of attraction between them. These forces are most likely to operate at the surface of each globule, in which case they will

[1] Doan, 1927, *J. Dairy Sci.*, 10, 501. 1929, *J. Dairy Sci.*, 12, 211.

be increased by increasing the exposed surface area of the globules. So one would expect the smaller globules to show greater attraction for each other than the larger ones normally present in fresh milk. But the colloids of milk are known to lower the surface forces, and it is also known that adsorption of the milk colloids takes place upon the surface of the globules. So it arises that the increased surface energy caused by homogenisation is largely diminished by the proteins present, and actual coalescence of the globules is rendered improbable on account of the adsorbed layer of proteins. If a cream of low fat content, or milk be homogenised, the globules, being relatively far apart, lose much of their surface energy by adsorption of proteins; but if the fat content be higher, then this increased surface energy will be manifested in the clustering of near neighbours before any extensive adsorption can cause such appreciable reduction of surface energy. The clusters so formed will subsequently adsorb protein on their outer surfaces.

In homogenised milk the increased adsorption at the fat globule surface renders the milk more viscous than raw milk. That the process of sub-division of the fat globules of cream should lead to increased viscosity may at first seem anomalous in view of the fact that the larger the fat globules the thicker is the cream. No doubt the explanation lies in the formation of clusters each with their absorbed protein layers. Some kind of structural arrangement of these clusters is responsible for the increased viscosity and the remarkable cream lines obtained when homogenised cream is diluted with separated milk.

The work of Orla Jensen supports the cluster theory of creaming.[1] He concludes that there exists in milk a substance agglutinin which by its action on the globule surfaces causes them to aggregate. He observes that the fat globules of heated milk may pass very close to one another or may even collide without showing any tendency to cluster. Although clustering may take place over a fairly wide range of temperature it is most favoured by comparatively low ones, namely, 7–8°C. of 45°F. Creaming therefore takes place in two stages: firstly, the clustering of fat globules, and secondly, the rising of these clusters strictly according to Stokes' law.

At low temperatures the viscosity of fat-free milk is higher, the difference in density between this liquid and the fat is lower, and the radii of the globules is lower, all of which effects would,

[1] Orla Jensen, 1928, *Proc. World's Dairy Congress*, p. 107.

according to the formula, *delay* creaming. The fact that comparatively low temperatures *favour* the formation of a good cream line in spite of the adverse effects on viscosity density, etc., serves to indicate how completely fat clustering dominates all other factors.

By heating milk to temperatures above 60°C. the creaming properties are reduced, and Orla Jensen attributes this to the progressive inactivation of agglutinin. He found that the addition of small amounts of the globulin fraction of milk was able to restore the creaming powers of heated milk, provided that the heating temperature had not exceeded 95°C. The association of agglutinin with the globulin of milk was noticed by Rowland in his work on the denaturation of the soluble proteins by heat (page 106).

Sharp and Krukovsky[1] have demonstrated that the physical state of the globules determines whether or not clustering may take place. Agglutinin is adsorbed by the semi-solid globules, but not by liquid ones, with the result that the former will cluster whereas the latter will not. They also confirm previous observations on the heat inactivation of this substance. A remarkable concentration of agglutinin was achieved in the following manner

(*a*) Cold milk was allowed to cream and the plasma withdrawn from the lower layers. The cream containing absorbed agglutinin was separated at 5–10°C., causing a further concentration in the cream fraction.
(*b*) This cream was then separated at 50°C., whereby the agglutinin was concentrated in the separated milk.

When this separated milk was united with cream which was separated from milk at 50°C., to give milk of 3·8 per cent fat, a cream volume of approximately 80 per cent was obtained two hours after the milk was formed. After twenty-four hours it had contracted to 35 to 45 per cent. Normal milk gave a 30 per cent cream volume two hours after setting. Combining the same hot-separated cream with a cold-separated plasma to give milk of the same fat content, but in which there would be very little agglutinin, resulted in a 10 per cent cream volume after two hours setting.

These experiments have furnished the explanation of the low temperature stimulus to the creaming of milk and have strengthened the cluster theory very considerably. Other theories of

[1] Sharp and Krukovsky, 1939, *J. Dairy Sci.*, 22 743.

creaming have been advanced from time to time, but it is not proposed to enter into a discussion of their merits, for although the cluster theory cannot yet be regarded as final, it does explain most of the facts.

The Mechanical Separation of Cream.—When milk is separated by mechanical means, the force of gravity is replaced by centrifugal force, and with this modification Stokes' equation is found to apply. As already mentioned, with increasing temperature, the difference in density between the fat and plasma also increases, because, although both are rendered less dense by expansion, the density of the fat decreases at a greater rate than that of the plasma. The increase per degree rise of temperature in the difference $(d_s - d_f)$ is greater up to 30°C., than beyond this point. Also the viscosity of the plasma decreases more rapidly between 5°C. and 40°C. than from 40°C. to 80°C. As the fat globules expand on heating the value r^2 also increases. From these considerations it is clear that at higher temperatures the efficiency of separation will increase to a marked degree. The increase in efficiency per degree rise of temperature is at a maximum at about 90°F. to 100°F. (32°C. to 38°C.) which are the temperatures commonly employed when most complete separation of fat is the object in view.

Separation of milk at lower temperatures will give a more viscous cream, but the efficiency of separation is much lower, considerable quantities of fat passing into the separated milk. The importance of cream viscosity in American dairy industries may be judged from the large numbers of research bulletins which deal with the subject. Although in this country the matter has not assumed such importance, it is probable that when conditions return to normal there will be a stimulus to the production and sale of various types of cream. Those interested in the technical details of this aspect of dairying are recommended to consult *Market Milk Industry*, 1941, by Roadhouse and Henderson, Chapter XVIII, page 345. One interesting development will be mentioned. Hening and Dahlberg[1] found that when pasteurised cream at 40°F. was warmed to 80°F. in an internal tubular heater and then recooled to 40–48°F., there was a very substantial increase in viscosity. The times of heating and cooling were approximately equal and ranged from three to ten minutes. By a combination of the two factors, fat content and temperature

[1] Hening and Dahlberg, 1932. *N.Y. Agric. Expt. Sta. (Geneva) Tech. Bull.*, 197.

treatment, it is possible to prepare creams of widely different viscosities. Several patents have been granted for various technical features of temperature treatment. A number of theories have been advanced to explain the temperature effect, but none seems entirely satisfactory.

Reconstituted (Artificial) Cream.—By vigorous emulsification of unsalted butter into milk, separated milk, or liquids reconstituted from the dried or condensed products, the fat globules are dispersed and coated with a layer of adsorbed protein, with the result that the product closely resembles cream. Machines of various capacity designed for this process of emulsification are on the market, and there is no doubt of the popularity of this cream 'substitute' whether in the household, in hotels, or aboard ship. The great advantage of reconstituted cream lies in the fact that it may be prepared when required and that the raw materials —butter, dried or condensed milk or separated milk possess such good keeping qualities. One characteristic of the product is the manner in which any flavour of the butter becomes intensified in the cream. A slight saltiness renders the cream unpleasantly saline and a "buttery" flavour so desirable in butter may spoil the cream. For these reasons the butter used should be as insipid as possible.

The Artificial (Reconstituted) Cream Act, 1929, requires that the name should be clearly stamped on all containers in which or from which it is sold and, further, that the name implies an article of food resembling cream and containing no ingredients not derived from milk, except water.

Synthetic Cream.—The substitution of margarine fat for butter fat in the above process will also give a valuable cream substitute. It may be distinguished from ordinary or reconstituted cream by the methods employed to distinguish butter from margarine.

Clotted Cream.—In the preparation of clotted cream, milk is allowed to form a cream layer by 'setting' in shallow pans for about twelve hours, after which it is scalded for fifteen minutes at about 187°F. Considerable evaporation occurs from the cream layer and on cooling over a period of twenty-four hours, the clotted cream may be removed by means of a suitable skimmer. The yield is about one pound of clotted cream per twenty-three pounds of milk. Although the product shows some variation in composition, the following analysis indicates its main characteristics:

	Per cent
Fat	63·00
Water	29·50
Protein	4·03
Lactose	2·80
Ash	0·67

Apart from its distinctive flavour, the main differences between clotted and ordinary cream are the increased ratio S.n.F./water of the former, namely, 1/4, and its relatively higher proportion of protein. Evaporation will certainly account for some of the increase in the S.n.F./water ratio and a concentration of protein at the liquid-air surface coupled with evaporation may well account for the relative increase in protein content.

Ice-cream.—On account of the growing popularity of ice-cream in this country and its well established demand in others, particularly the United States of America, it is proposed to include some account of its general composition. The reader desiring further information regarding its small scale or commercial preparation is referred to *The Making of Ice-cream*, by R. M. Feltham, 1934, published by Heywood & Co., London; also to pre-war, and it is hoped post-war, issues of *Dairy Industries*, London.

The raw materials may be drawn from milk, cream, butter, sweetened condensed milk, powdered milk or separated milk, sugar, gelatin, lecithin or egg yolk preparations, sodium alginate, and flavouring essences. Rodgers[1] gives the following ranges of composition for ice-cream mixes:

	General range	Composition of a typical ice-cream mix
	Per cent	Per cent
Fat	8–18	12
Milk S.n.F.	6–12	10
Sugar	13–17	14
Gelatin	0·25–0·50	0·3
Total solids	30–40	36·3
Water	70–60	63·7
Freezing-point		−2·26°C.

Given a mix of satisfactory composition, the quality of the product is largely determined by its texture, which must be

[1] Rodgers, 1928, *Fundamentals of Dairy Science*, p. 229.

smooth and open. Several factors combine their influence in this respect, amongst them being the rate of freezing, the temperature of the freezing medium, and the mechanical whipping of air into the mixture by means of beaters. By this latter means there is a considerable increase in volume and a consequent open texture resulting from the stabilisation of air bubbles by protein absorption. Without this whipping process the product would be heavy or soggy. Generally the milk S.n.F. must be kept below 10 per cent in order to avoid the grittiness caused by crystallisation of lactose. Gelatin improves the smoothness of the product, increases its resistance to melting and in general acts as a stabiliser. Sodium alginate, a substance obtained from alginic acid of seaweed, is considered superior to gelatin in this respect. Lecithin or egg yolk preparations are valuable emulsifiers. On a large scale the mix is pasteurised, and then homogenised to prevent fat churning and to improve the smoothness of the product. After freezing and whipping, the ice-cream is transferred to hardening chests kept at —10°C. or lower, whereby crystallisation is carried further and the product hardens.

A practical problem often arises where a mix of given composition has to be compounded from dairy products whose composition varies. In such cases these products would need analysing for fat or milk S.n.F., when the correct proportions can be calculated. By way of example suppose it is desired to compound a mix containing 10 per cent fat, 10 per cent milk S.n.F., 14 per cent sugar, and 0·5 per cent gelatin. Sugar, gelatin, milk, cream, and separated milk powder are available. The milk, cream, and separated milk powder are known to contain:

	Fat	S.n.F.
	Per cent	Per cent
Milk	3·5	8·75
Cream	50	4·5
Separated milk powder .	—	96·5

In every 100 lbs. of mix, let a = lbs. of cream, b = lbs. of separated milk powder, and c = lbs. of milk. As the mix must contain 14 per cent sugar, and 0·5 per cent gelatin, 100 − 14·5 = per cent dairy products = 85·5.

$a+b+c=85{\cdot}5$ . . . . . Equation 1

a lbs. of cream will give 0·5a lbs. of fat.

c lbs. of milk will give 0·035c lbs. of fat.

therefore, as the mix must contain 10 per cent fat we have,

$0{\cdot}5a+0{\cdot}035c=10$ . . . . . Equation 2

a lbs. of cream will give 0·045a lbs. of milk S.n.F.

b lbs. of separated powder will give 0·965b lbs. of milk S.n.F.

c lbs. of milk will give 0·0875c lbs. of milk S.n.F.

As the mix must contain 10 per cent of milk S.n.F., it follows that

$0{\cdot}045a+0{\cdot}965b+0{\cdot}0875c=10$ . . . . Equation 3

From equation 2

$0{\cdot}5a=10-0{\cdot}035c$

$a=20-0{\cdot}07c$

Substituting for a in equation 1 we have:

$(20-0{\cdot}07c)+b+c=85{\cdot}5$

$b+0{\cdot}93c=65{\cdot}5$ . . . . Equation 4

Substituting for a in equation 3:

$0{\cdot}045\ (20-0{\cdot}07c)+0{\cdot}965b+0{\cdot}0875c=10$

$0{\cdot}9-0{\cdot}00315c+0{\cdot}965b+0{\cdot}0875c=10$

$0{\cdot}965b+0{\cdot}08435c=9{\cdot}1$ . . Equation 5

multiply equation 4 by 0·965:

$0{\cdot}965b+0{\cdot}89745c=63{\cdot}208$

subtract equation 5 we have:

$0{\cdot}8131c=54{\cdot}108$

$c=66{\cdot}54$

From equation 2 $a=20-0{\cdot}07c$

$=20-4{\cdot}658$

$=15{\cdot}342$

From equation 1 $b=85{\cdot}5-(a+c)$

$=85{\cdot}5-81{\cdot}88$

$=3{\cdot}62$

The mix is therefore made up as follows:

Cream	15·34 lbs.
Separated milk powder .	3·62 lbs.
Milk	66·54 lbs.
Sugar	14·00 lbs.
Gelatin	0·50 lbs.
	100·00 lbs.

The calculation may be checked as follows:

	Fat	Milk S.n.F.
15·34 lbs. of cream supply	7·67 lbs.	$15{\cdot}34 \times 0{\cdot}045 = 0{\cdot}690$ lbs.
3·62 lbs. of sep. powder give	—	$3{\cdot}62 \times 0{\cdot}965 = 3{\cdot}483$ lbs.
66·54 lbs. milk supply	$66{\cdot}54 \times 0{\cdot}035 = 2{\cdot}32$ lbs.	$66{\cdot}54 \times 0{\cdot}0875 = 5{\cdot}822$ lbs.
	Total 9·99 lbs.	Total 9·995 lbs.

Whipped Cream.—When cream of 36–40 per cent fat is whipped, the incorporated air bubbles are stabilized by protein adsorption. Around these bubbles the fat clusters assemble as the process of whipping proceeds. In this manner the air bubbles first included are stabilised and, as whipping continues they are sub-divided into smaller nuclei each with an adsorbed layer and upon the surface of each, innumerable fat clusters aggregate. The whole process is very similar to the formation of butter grain nuclei during the churning of cream, and the analogy is completed by the fact that overwhipping produces a buttery product. At the maximum stiffness which is the desired aim of whipping, these nuclei are surrounded by films of absorbed protein which are so thin that drainage is at a minimum and the structure of a dry foam is set up.

The aggregation of fat clusters around the air bubbles is best effected when the fat is in the semi-solid state. There is considerable evidence to show that the success of whipping depends on the formation of clusters in the cream. Temperatures below 10°C. which are known to be conducive to clustering are also found to be most favourable to whipping. Conditions detrimental to clustering are detrimental to the process of whipping. In so far as the ageing of cream favours clustering, it also assists whipping. Homogenisation is detrimental to whipping in spite of the fact that the fat globules of homogenised cream are in the clustered form. This is yet another indication of the peculiar structure set up in cream by homogenisation.

Butter.—A superficial comparison of the physical properties of cream and butter will give some impression of the profound changes brought about by the process of churning. More detailed microscopic examination will reveal the existence of two phases in each product. In cream, water is continuous and fat the dispersed phase, whereas in butter the fat is continuous and water droplets are dispersed therein. It is not surprising that this phase inversion should have been the subject of many ingenious theories. While

none is absolutely satisfactory the generally accepted modern view has formed a useful working hypothesis. It may be considered as an extension of the changes taking place in the whipping of cream:

(*a*) The mechanical incorporation of air bubbles, and

(*b*) Their protection by an adsorbed layer of protein,

(*c*) The aggregation of fat clusters around the liquid—air surfaces, and

(*d*) Further sub-division of these particles.

But whereas whipping requires a cream of about 38 per cent fat, and stops at the point of maximum stiffness, churning is most successful with cream of 29 to 35 per cent fat, and continues the process of piling fat clusters around these minute nuclei until the ratio surface area to volume becomes low enough to overcome the friction of the surrounding liquid, whereupon the grains rise to the surface and the butter 'breaks.'

The physical condition of the fat has a profound influence on churning, which may be successfully carried out only when the fat is in a semi-solid condition. Neither completely liquid nor fully solid fat are favourable to the process, and it follows that the extreme range of churning temperatures must lie between the melting- and setting-points of the fat. In practice the temperature of churning varies between approximately 50°F. and 65°F. according to season, locality, and previous treatment of the cream. Owing to the time-lag in the adjustment of the physical condition of the fat to new temperatures, the churning temperature of a recently cooled cream must be lower than that of a cream which has been warmed recently to the churning temperature. Similarly, when cream is held for different lengths of time at low temperatures, the churning temperature is higher the longer the cream is stored.

It has been observed in the making of farmhouse butter that milk from cows in advanced lactation may give cream which is difficult to churn. This is generally ascribed to the liberation by lipolysis of fatty acids which lower the surface tension and cause excessive foaming; and to the smaller diameter of the globules.

The rise of temperature which occurs during churning may be due to a number of causes chief among which, in the opinion of the author, is the heat generated by friction of the cream against the walls of the churn.

In addition to the incorporation of minute air bubbles, churning causes minute water droplets to be embedded in the grains where they are stabilised by adsorbed proteins. Water also adheres in much larger drops to the surface of the grains and much of this is expelled in the working operations. Satisfactory working leaves the water evenly distributed throughout the mass in the form of minute droplets which, by their scattering effect on light, tone down of the yellow colour of the fat to the more characteristic opaque creamy colour. Mottled or streaky effects are due to faulty working causing uneven distribution or abnormal subdivision of the water droplets. The number and size of these droplets have formed an interesting study. They range from 1·9μ to over 100μ about half the water content of butter being distributed amongst droplets less than 15μ diameter. The total number of droplets per gramme of butter is enormous, running into billions. As this number far exceeds the number of micro-organisms it follows that the majority of the droplets are sterile. However, this does not mean that the water droplets are necessarily chemically inert. Indeed, it is the absorbed layer at the water-fat surface which brings metals like copper into close proximity with the fat, and which, together with the dissolved oxygen within the droplet, brings the mechanism of fat oxidation into action. While it may be argued that the dispersion of water in butter affords some protection against bacterial deterioration it is also true that by increasing that interfacial area this dispersion favours fat oxidation. It is the view of some workers that overworking may predispose the butter to oxidative taints through the incorporation of air pockets.

Butter Flavour.—The desirable flavours associated with good butter are mostly derived from the cream, and on this account alone, the ripening of cream prior to churning must be conducted under carefully controlled conditions. It is true that raw cream contains micro-organisms which, given favourable conditions, will initiate lactic fermentation with the production of flavours as by-products. The results by this method are very erratic and many years ago it became apparent that the best and most reproducible results were to be obtained by inoculation of the cream with cultures known to give the most desirable flavours. As early as 1890, commercial butter cultures were used for cream ripening, but only comparatively recently has their action received any measure of elucidation.

One of the flavouring agents produced in cream ripening is diacetyl, $CH_3CO.COCH_3$. It possesses a most powerful buttery odour and taste and is produced by oxidation of its precursor, acetyl methyl carbinol.

$$CH_3CHOH.COCH_3 + O = CH_3CO.COCH_3 + H_2O$$

or by the reaction

$$2\,CH_3CHOH.COCH_3 = \underset{\text{Diacetyl}}{CH_3CO.COCH_3} + \underset{\text{Butyleneglycol}}{CH_3.CHOH.CHOH.CH_3}$$

So powerful is this flavouring essence that as little as one part per million in butter will impart a distinctive flavour. The following figures will illustrate this point further.[1]

Flavour of Butter	Concentration of diacetyl in butter Parts per million	Acidity of 35% fat cream to produce the flavour	
		Per cent	
Flavourless	0 –0·3	0·1 –0·15	as lactic acid
Mild	0·5–1·5	0·15–0·25	
Medium	1·0–3·0	0·25–0·40	
Full	3·0–5·0	0·40–0·75	

These figures must not be interpreted as meaning that the attainment of the requisite degree of acidity will automatically produce the desired flavour. Indeed, one essential of a good butter culture is that the flavour-producing organisms (*S. citrovorus* and *S. cremoris*) should be able to proliferate before the acid producers remove dissolved oxygen to such a degree that the flavourless acetyl methyl carbinol is not oxidised to diacetyl. The flavour-producing organisms in a good butter culture are carefully balanced against the acid formers.

Considerable losses of diacetyl and its precursor occur in the making of butter as, being more soluble in water than in fat a very large proportion of them is run off with the buttermilk. While both are equally water-soluble the diacetyl is more soluble in fat than is its precursor acetyl methyl carbinol. The retention of the former by butter (9 per cent of total) is therefore greater than that of the latter (4 per cent of total), and on this account the maximum flavouring effect is secured under conditions of cream ripening which favour the production of diacetyl rather than its precursor.

[1] W. L. Davies, 1938, *Agric. Progress*, 15, 30.

Dissolved oxygen in cream may become a limiting factor in the formation of diacetyl, but the incorporation of air during churning and working allows further oxidation of acetyl methyl carbinol to proceed. For this reason the full flavour of butter appears 12 to 24 hours after churning.

Opinions differ regarding the source of diacetyl and its precursor. Some consider that both are produced from lactose while others claim that they are obtained from bacterial attack on the citrates of milk. Prill and Hammer[1] favour the latter view having observed that addition to milk of 0·15 per cent citric acid improved the flavour of starters. In most cases inoculation of cream with these improved starters gave better-flavoured butters. Davies, however, considers that addition of citric acid merely favours the growth of the flavour-producing organisms and that lactose is the source of diacetyl.

Some Defects in Butter Flavour.—It is common experience that a full-flavoured butter produced from a high acid cream is far more prone to fat deterioration than the milder types obtained from moderate ripening. If cream is allowed to develop high concentrations of lactic acid, some of this compound is dissolved by the fat, which then becomes more susceptible to oxidative changes leading to fishiness and tallowiness. This condition is further aggravated by metallic contamination.

Davies[2] studied the effect of metallic contamination by adding various quantities of metallic lactates during the salting of butter. Some of his results are as follows:

Copper added, 1 part per million of butter.
Butter stored at 15°C. was fishy in 60 hours and tallowy in 100 hours.
,, ,, 0°C. ,, ,, 72 ,, ,, ,, 120 hours.

The butter was in a state of advanced rancidity in one week. With 2 p.p.m. of copper the fishy taint appeared in 48 hours and the tallowy taint in 72 hours. Contamination with 10 p.p.m. of copper caused the tallowy taint to appear so quickly that no fishiness could be detected. Acidity in all cases hastened these changes. As shown in the table, the effects of Nickel, Manganese, Chromium, and Iron were not so severe as those of copper, but here again they were hastened by acidity. In addition to its effect on flavour, fat oxidation is known to cause losses of vitamin A.[3]

[1] Prill and Hammer, 1939, *J. Dairy Sci.*, 22, 67.
[2] W. L. Davies, 1937, *Proc. 11th World's Dairy Congress*, Berlin, 2, 124.
[3] Hilditch, 1944, *Chem. and Ind.* p. 67.

Concentration.	Time in days for taint to develop	
Parts per million	Fishy	Tallowy
5	5	9
10	4	8
25	3	7

The wrapping material of butter may, by its absorption of water cause a concentration of the contaminating metal and intense localised oxidation may ensue.

Rancidity in butter is generally believed to be due more to the lipolytic activity of bacteria and moulds rather than to the natural lipase of milk. The subject has received much attention, and while there is general agreement that the initial reaction is one of hydrolysis whereby free fatty acids and glycerol are liberated from the fat, there are indications that the fatty acids of glycerol, or indeed both, may suffer further changes which lead to the production of the typical odours and flavours of rancid butter. If the substances responsible for rancidity are fatty acids, the acid value of the fat should increase as the taint develops. Some rancid butters have quite normal acid values and some normal butters are found to have high acid numbers. This aspect of the subject has been examined by Fouts[1] whose papers should be consulted for further details. Our knowledge of the mechanism of rancidity is very incomplete, but it is clear that in the case of butter, it is not simply a matter of the liberation of odoriferous fatty acids. Davies[2] describes ketonic rancidity of butter which is caused by 'dry' moulds acting on the surface with the liberation of methyl ketones from the fatty acids. Much can be achieved in the avoidance of butter defects by pasteurisation of the cream and thorough washing of the butter grains with clean water. The use of wrapping parchment which has been soaked in propionate solutions has been advocated for suppressing mould growth.

Cream Neutralisation.—This is a matter which assumes much greater importance in other countries, but there may be occasions when a knowledge of the fundamental principles of the process will prove useful. When cream is transported to a central butter-making station it may arrive in too acid a condition to allow of satisfactory pasteurisation, or the high acidity may cause the

[1] Fouts, 1940, *J. Dairy Sci.*, 23, 173, and 245.
[2] Davies, 1939, *The Chemistry of Milk*, p. 89.

butter to have poor flavour and texture. As already stated, butters made from highly acid creams usually possess poor keeping qualities.

Neutralisation cannot eliminate all these disadvantages, but it does reduce them to a minimum. It aims at reducing the acidity to 0·1–0·3 per cent lactic acid, the lower values to apply where, after pasteurisation, the cream is to be ripened with butter cultures.

The student probably thinks of neutralisation in terms of alkalis such as sodium hydroxide, but this substance is never used for cream neutralisation owing to its powerful action and the dangers of over neutralisation. Hydrated lime may be used, but it suffers from the disadvantage that it is not very soluble in water. Further, although it may be added in strictly theoretical quantities (37 parts by weight neutralise 90 parts by weight lactic acid) the actual neutralising power is only about 80 per cent of theory. Sodium carbonate and bicarbonate have the advantage of being soluble and of bringing about the theoretical reduction of acidity provided steps are taken to remove the liberated carbon dioxide gas. Sodium carbonate may be purchased as anhydrous Na_2CO_3, or as crystalline Na_2CO_3, 10 H_2O. The former absorbs water and the latter loses it when exposed to the atmosphere. They must on this account be stored in air-tight containers and the lid replaced immediately after use.

Equations for the neutralisation of lactic acid with these two substances are as follows:

$$2\,CH_3CH(OH)COOH + Na_2CO_3 = 2\,CH_3CH(OH)COONa + CO_2 + H_2O$$
$$CH_3CH(OH)COOH + NaHCO_3 = CH_3CH(OH)COONa + CO_2 + H_2O$$

The molecular weights of lactic acid, anhydrous sodium carbonate, and sodium bicarbonate are respectively 90, 106, and 84. It follows that 1 lb. of lactic acid is neutralised by $\frac{84}{90} = 0{\cdot}93$ lb. sodium bicarbonate, or $\frac{106}{180} = 0{\cdot}59$ lb. anhydrous sodium carbonate. The molecular weight of sodium carbonate crystals is 286, from which it follows that 1 lb. of lactic acid would require $\frac{286}{180} = 1{\cdot}59$ lbs. for complete neutralisation.

Suppose, for example, that a sample of cream has an acidity of 0·55 per cent, and that it is required to reduce it to 0·15 per cent.

Every 100 lbs. of cream must lose $0{\cdot}55-0{\cdot}15=0{\cdot}40$ lb. of lactic acid. If sodium bicarbonate is to be used it will be necessary to use $0{\cdot}4\times0{\cdot}93=0{\cdot}372$ lb. (approximately 6 ozs.), if neutralised with anhydrous sodium carbonate $0{\cdot}4\times0{\cdot}59=0{\cdot}236$ lb. (3·75 ozs.) would be required, or $0{\cdot}4\times1{\cdot}59=0{\cdot}636$ lb. (approximately 10 ozs.) of sodium carbonate crystals Na_2CO_3 10 H_2O.

Carbon dioxide gas being slightly soluble, the full reduction of hydrogen ion concentration will not be effected until pasteurisation removes some of this gas. Hunziker[1] suggests that the required amounts of sodium carbonate (anhydrous) or sodium bicarbonate should be weighed and dissolved in water to make a 10 per cent solution which is sprinkled on the surface of the cream with constant agitation. There is a tendency for highly acid creams to froth when the bicarbonate is used, but this may be overcome by dissolving in hot water and adding the solution hot. Some authors[2] consider it desirable to warm the cream to 80–90°F., and to add the neutralising solution to the warm cream.

Over neutralisation may cause difficult churning and may produce a soapy butter of poor texture and unsatisfactory keeping qualities. Neutralisation must therefore be carried out with the utmost care, and its success will largely depend upon the accuracy with which the acidity of the cream is determined. Even when satisfactorily performed it is admitted that butter made from neutralised cream does not possess the keeping qualities of ripened sweet cream butter. One reason is that although the process may neutralise the bulk of water soluble lactic acid, it does not effect a proportionate reduction in the fat soluble acid.

The influence of salt on the keeping quality of butter is a matter of interest for while salt is antagonistic to most bacteria and moulds, the common experience has been that butters containing over 2 per cent of salt do not keep so well as slightly salted ones. One possible explanation for this may be the increased solubility of lecithin in salt solutions whereby more of this compound enters the aqueous phase, where acidity and dissolved oxygen favour its oxidation.

Badly flavoured butter may occasionally be rendered edible by melting, and washing the fat with warm water in order to remove the products of decomposition as far as possible. The fat may then be emulsified into milk or separated milk. This

[1] Hunziker, 1927, *The Butter Industry*, p. 168.
[2] Totman, McKay, and Larsen, 1939, *Butter*, p. 122.

product, named milk-blended butter, is to most people a curiosity mentioned in the Sale of Foods and Drugs Act where it is stated that a maximum of 24 per cent water is allowed. The limit for butter and margarine is 16 per cent water.

The composition of butter varies and a few analyses are given in the following table.

	Author								
	Vieth[1]	Hunziker[2]		Cox[3]					Ling[4]
Fat . .	86·85	81·5	83·5	86·82	84·94	81·97	82·22	81·79	88·6
Water .	11·54	15·0	15·2	12·15	14·26	15·03	11·74	14·00	12·5
Non-fatty solids (excluding salt) .	0·59	1·0	1·3	1·00	0·75	0·70	1·01	1·31	0·7
Salt . .	1·02	2·5	0·0	0·03	0·05	2·30	5·03	2·90	0·2
	100·00	100·00	100·0	100·00	100·00	100·00	100·00	100·00	100·0

Margarine.—The first butter substitute owed its creation to the dire necessities imposed upon the French by the Franco-Prussian war, which caused Napoleon III to offer factory facilities and a substantial reward to the inventor. The prize was won by Mège Mouriés who aimed at making butter by a process of gastric digestion of animal fats. The records of his experiments whereby animal fats were warmed with macerated sheep's stomach and chopped cow's udder are strongly reminiscent of the early alchemical writings. He was convinced that he had made butter but his process was in effect a separation by thermal treatment of the softer fats from such animal fats as were available to him. The idea of producing butter substitutes quickly spread to Holland, United States of America, and Great Britain. In the latter, 'factories' sprang up in most unsavoury premises and the equally unsavoury product earned an evil reputation which has prejudiced the industry ever since; notwithstanding the vastly different conditions of manufacture. The first great war also caused a set-back for the industry owing to flavour defects arising from serious limitations of supply of suitable raw material.

[1] Vieth, quoted by Richmond's *Dairy Chemistry*, 1942, p. 165.
[2] Hunziker, 1927, *The Butter Industry*, p. 450.
[3] Cox, 1938, *The Analysis of Foods*, p. 271.
[4] Ling, unpublished data.

At the time of writing margarine enters largely into our war-time diet and, in the author's opinion, an excellent standard has been maintained in spite of limitations of supply. The second great war has seen margarine come into its rightful place. The modern product relies mostly on vegetable oil seeds as sources of fat and upon ripened separated milk as a flavouring agent. Ground nuts (monkey nuts), coco-nuts or copra, palm kernels, and soya beans are all able to furnish oils which are used in making margarine. These oils are extracted from the seed and the residues provide valuable oil cakes or meals for stock-feeding. The oils are decolorised, deodorised, and purified to such an extent as to be practically without taste. They are then blended to give a mixture having the same melting and setting ranges as butter fat. Climatic conditions are also considered in the blending process. Oils from the soya bean and ground nut are liquid, but they may be rendered solid or semi-solid by the fat hardening (hydrogenation) process:

unsaturated liquid fat + hydrogen = more saturated semi-solid fat.

Liquid ground nut oil, for instance, may be hardened by this process to give a fat melting at 35°C., or even higher if required. This highly technical process has placed a much wider range of oils at the disposal of the margarine manufacturer: a matter of great importance in time of war.

The successful making of margarine also depends upon the development of butter flavours in pasteurised separated milk by inoculation with butter cultures, which are generally propagated in the bacteriological laboratories attached to the works.

The oils are homogenised into the ripened separated milk in the proportion of 80 per cent oil to 20 per cent separated milk. The temperature of emulsification varies between 25–40°C., according to circumstances. Concentrated preparations of vitamins A and D are added at this stage. The emulsion flows on to the surface of a revolving drum which is cooled internally to a temperature of −15°C. An exceedingly thin film of frozen emulsion is removed from the drum by a scraper and conveyed to a maturing room where it is left some hours at ordinary temperature. During this time the fat absorbs the flavours produced in the ripened separated milk and its physical condition slowly adjusts itself to the new temperature. It is then worked like butter, again allowed to mature and then thoroughly mixed

in a large blender. At this stage the moisture content is checked and, if required, egg yolk preparations are added to give the margarine desirable "browning" properties. Blending with butter is permitted by the Foods and Drugs Act up to a maximum of 10 per cent butter fat. Weighing, wrapping, and packing are performed mechanically. The following table summarises the chief points of resemblance or difference between butter and margarine.

Butter	Margarine
An emulsion of water in butter fat produced by the churning of cream.	Possesses similar physical structure but is produced by emulsification of vegetable oils in ripened separated milk.
Must contain less than 16 per cent water.	16 per cent water maximum limit.
Flavour derived from ripening of cream by butter starter.	Flavour largely derived from ripening of separated milk by similar cultures.
Vitamins A and D vary with the season of year, feeding of the dairy herd, etc,	May be vitaminised to a steady all the year round level.
Two by-products, separated milk and buttermilk.	Production closely linked with the oil cake industry.
Produced from perishable raw materials.	Raw materials except separated milk have long storage life. The separated milk could be made from the powder if necessary.
Fat is a mixture of glycerides of the typical milk fatty acids.	Fat a mixture of glycerides in proportions different from those of butter fat. Some of the milk fatty acids may be missing on occasion.
Fat contains the animal sterol cholesterol.	Fat contains the vegetable sterol phytosterol.
Physical nature of the fat is influenced by seasonal and feeding factors.	Physical nature of the fat is under complete control.

The composition of margarine varies somewhat according to market requirements, but the following may be accepted as a

typical sample: Fat, 84·5 per cent; Non-fatty solids excluding salt, 1 per cent; Salt, 1·5 per cent; Water, 13·0 per cent. From the similarity of these figures with those of butter, it is obvious that discrimination between the two products or the detection of the presence of margarine in butter must involve a much more thorough analysis. For this purpose it is necessary to separate the fat and examine its chemical characteristics. The proportion of steam volatile fatty acids in margarine fat will be very different from that of butter fat, so will their distribution between the water soluble and insoluble phases differ. Differences in the saponification value, iodine value, etc., will also help to discriminate between the two fats, and the presence of phytosterol will establish with certainty the presence of vegetable oils.

CHAPTER VI

CHEESE, ANNATTO, AND SOFT CURD MILK

Cheese.—The origin of this valuable food is unknown, but it was certainly made in very early times for there are references to it in the earlier books of the Old Testament. It is probable that the first cheese was made from curds obtained from the natural souring of milk and that the discovery of rennet cheese arose from the carrying of milk in a stomach "skin" in which the stomach enzymes were still active. Whether made from sour milk curd or by the action of stomach extracts, the importance of the process as a means of conserving much of the food value of milk in a product which was much less perishable was quickly appreciated, particularly by peoples in warmer latitudes. From either method of production, the whey was used as a human beverage, but while civilisation has deepened man's appreciation of cheese it is a matter of regret that it has deprived him of his early liking for whey.

Certain plants furnish enzymes capable of coagulating milk. Owing to a tendency to produce bitter cheese their use in cheese-making has not been widespread. Butterwort (*Pinguicula vulgaris*), Lady's Bedstraw (*Galium verum*), and the seeds of *Withania coagulans* have been used as sources of vegetable 'rennets.' Some bacteria are able to clot milk, not by the acids they produce, but by enzymes secreted by their cells.

The coagulation of milk by rennet is so widespread that a brief account of the preparation and characteristics of this enzyme forms a necessary preliminary to a study of cheese. The stomach secretes two enzymes which are capable of coagulating milk under the acid conditions which characterise stomach secretions. Rennin predominates in the stomach of the very young animal and pepsin increases as the animal matures. The two enzymes are similar in many respects, so similar that they were believed to be identical, but it is now known that their activity is governed by different conditions. For instance rennin is able to coagulate milk of very low hydrogen ion concentration and its optimum *p*H is in the region of 5·4, whereas pepsin is able to act at much

higher acidities which are unfavourable to rennin. Further it is known that rennin is able to break down the protein molecules to a much greater extent than pepsin, indeed one disadvantage of the presence of pepsin in rennet extracts is the tendency to produce peptones in cheese. These are believed to give a bitter flavour to the ripened product. The fourth stomach of young calves or occasionally the digestive stomachs of sheep or pigs are used as sources of rennin. Although the enzyme is soluble in dilute salt solutions, the extracts are strongly saline in order to depress bacterial activity as far as possible.

Rennin is an acid protein of the globulin type whose activity seems to be closely connected with its acid nature. Unlike globulin, it may not be precipitated by heat although it may be inactivated thereby. Temperatures above 49°C. (120°F.) cause a progressive weakening of the enzyme. The familiar time-temperature relationship is again demonstrated. Struble and Sharp[1] inactivated rennin by heating to 50°C. for 14 minutes at a *p*H 6·8–7·0. Like other proteins it may be salted out of its solutions by ammonium sulphate. By vigorous shaking it becomes precipitated at the liquid-air surface and its activity is thereby reduced. In common with most enzymes it is very sensitive to conditions of temperature, acidity, and the presence of certain metallic salts. Optimum conditions are represented by a temperature 40–41°C. a *p*H in the region of 5·4, and the presence of calcium ions. Alkaline conditions are inhibitory.

Liquid rennets usually contain the enzyme pepsin in addition to rennin, and, moreover, they may not be entirely free from bacteria. Davis[2] points out that these organisms are not necessary for the production of good cheese and that they may be responsible for some of its faults. Of recent years the preparation of purified rennins has received much attention. Hankinson and Palmer[3] were able to separate the peptic enzymes from rennin by bringing liquid rennet extract to a *p*H 4·5 by addition of concentrated hydrochloric acid (7 ml. conc. HCl to 2 litres of rennet), whereby rennin was precipitated and the peptic enzymes remained in solution. The rennin was removed by centrifugal force, and by utilising its solubility in salt solution (20 gms. NaCl to 100 ml. water) and its precipitation at *p*H 4·5, further purification was effected. The red-brown colour of commercial rennets is removed

[1] Struble and Sharp, 1940, *J. Dairy Sci.*, 23, 587.
[2] Davis, 1938, *J. Dairy Res.*, 9, 80.
[3] Hankinson and Palmer, 1940, *J. Dairy Sci.*, 23, 520.

during these operations and it appears to be identified with the peptic fraction rather than with rennin. It is noteworthy that the iso-electric point of rennin is so nearly the same as that of casein. The corresponding point for pepsin lies in the region of *p*H 2·75. Berridge[1] has described the preparation of a pure crystalline rennin from the impure precipitate obtained by saturation of commercial rennet with sodium chloride at a *p*H 5·4.

It is possible to purchase purified rennins in either liquid or tablet form and results to date have proved them more reliable in cheesemaking than the more traditional rennets. The tablets store better than the liquid preparations. The activity of any rennin preparation is checked against a standard rennin by comparing the times of coagulation of the same sample of bulk milk, or a reconstituted spray dried milk. The technique of these rennet tests is fraught with many difficulties and those desiring more detailed information are recommended to consult some of the original papers on the subject.[2]

The Action of Rennet on Milk.—It will be of great assistance in studying the various circumstances which control rennet action if it is realised at once that the formation of a coagulum proceeds by two very well-defined stages,[3] namely the attack of rennin on the casein complex followed by coagulation of the major products of the first action. One difference between the two is that stage one is enzymatic while the second is not.

Stage 1. Enzymes have a temperature coefficient of 2–4 per 10°C., that is to say for each 10°C. rise of temperature the speed of the reaction is multiplied 2–4 fold. Stage 1 proceeds according to this rule. Conversely, every 10°C. fall in temperature will prolong the time taken for stage 1 to reach completion. At 0°C. the rate of reaction will be slow, but Berridge[4] has shown that it proceeds at a measurable rate which agrees with the calculated value. For instance a milk which at 37°C. completes stage 1 in 8 minutes should require 130 minutes at 0°C. Whereas a clot is formed in the former case none is obtained at 0°C., *because the second stage does not proceed at temperatures below* 10–20° C.

Stage 2. On account of this peculiar temperature effect, it is most unlikely that the second stage is merely one of precipitation.

[1] Berridge, 1943, *Nature*, 151, 473.

[2] Mattick and Hallett, 1929, *J. Agric. Sci.*, 19, 452; King and Melville, 1939, *J. Dairy Res.*, 10, 340; Sommer and Matsen, 1935, *J. Dairy Sci.*, 18, 741.

[3] Linderstrom-Lang, 1928, *Z. Physiol. Chem.*, 176, 76; Holter, 1932, *Biochem. Z.*, 255, 160.

[4] Berridge, 1942, *Nature*, 149, 194.

Preliminary experiments by Berridge have shown that at temperatures above the critical 10–20°C. range, the temperature coefficient is 1·3 to 1·6 per degree Centigrade. The only relevant reaction showing a similar temperature effect is that of protein denaturation. The hypothesis is advanced that stage 2 of rennin action is a process of protein denaturation, that is a regrouping of poly-peptide linkages within the protein complex, and it gains support from the well-known fact that increases in concentration of calcium ions cause marked acceleration of the formation of a clot. There are parallel instances of protein denaturation being stimulated by calcium ions. Very small additions of calcium equivalent to $\frac{N}{5000}$ in milk, were found by Berridge to have such a pronounced hastening effect on this stage that he came to the conclusion that the concentration of calcium ions normally present must be of a very low order.

Hankinson and Palmer[1] studied the action of rennin on calcium caseinate systems and came to the conclusion that the enzyme renders the casein unstable by virtue of a process of *dehydration*.

The change which occurs when rennin attacks milk may be written as:

$$Ca_3P_2O_8\,[\text{calcium caseinate}] \longrightarrow Ca_3P_2O_8\,[\text{calcium para-caseinate}] + \text{a proteose.}$$

Casein is converted into a new protein, para-casein, and at the same time a proteose is set free. The former remains in the curd while the latter is drawn off in the whey where it supplements the soluble nitrogen originally contained in the milk. Casein and para-casein differ very considerably in physical properties, but for a long time no chemical difference could be detected, until it was discovered that the calcium binding capacity of para casein was about twice that of casein. In milk, the base binding capacity of casein is nothing like satisfied, and it follows that para-casein is even more unsaturated towards calcium, a circumstance which may well be related to its sensitivity to calcium ions.

The proteose, which has been known for many years as Hammarsten's proteose, has been the subject of much controversy. It has been thought to be a product of peptic digestion of the rennet curd, but in the author's opinion this view must be discounted by the observation that it is formed quicker than one

[1] Hankinson and Palmer, 1943, *J. Dairy Sci.* 26, 1043.

would expect from peptic action and, moreover, the proportion of proteose as a percentage of casein nitrogen is very constant, namely 4 per cent. Some workers consider that it arises from a definite cleavage of the casein complex, while Cherbuliez and Jeannerat consider that it represents the fraction of the casein mixture that is not coagulated by rennet. If the latter conception is correct, then stage 1 of rennet action proceeds by disengaging from the casein mixture the soluble proteose which acts as a protective colloid, leaving the remaining fractions open to further attack by the enzyme. It is not yet possible to state which view is correct but the author considers that the latter holds promise of profitable investigation.

The time taken by rennet to coagulate milk depends upon the following factors:

(1) concentration of rennet; (2) temperature of the milk; (3) acidity; (4) the balance of soluble mineral matter; (5) previous treatment of the milk; (6) presence or absence of inhibitory substances.

Concentration of Rennet.—In common with other enzymes very small quantities of rennin are able to transform exceedingly large quantities of milk. For example Fenger[1] found that one part of rennet was able to coagulate in 10 minutes two million parts of milk at 40–41°C. In cheesemaking the proportion is about 1 in 5000. Within the normal temperature range the time of coagulation is claimed to be inversely proportional to the concentration of rennin. Dilution of milk increases the time of coagulation owing to reducing the concentration of rennin and to alterations in the ionic equilibria. Dilution lowers the concentration of soluble calcium salts, but it will also encourage ionisation. It is to be expected therefore that the adverse effect of lowered concentration of soluble calcium will be offset to some extent by increases in calcium ion concentration. This is precisely what does happen. Up to 10 per cent of water may be added to milk before the retarding effect of dilution is noticed.

Effect of Temperature.—As noted earlier in this section, stage 1 is characterised by a normal temperature coefficient whereas stage 2 does not proceed below 10–20°C. and a much greater temperature effect is observed. The optimum temperature for rennet coagulation, 40–41°C., must be regarded as a generalisation on

[1] Fenger, 1923, *J. Amer. Chem. Soc.*, 45, 249.

the resultant of these two independent changes. The following figures must be interpreted in the same manner.[1]

Temperature °C. . .	23	28	33	38	43	48
Time of coagulation in minutes . . .	36	11·1	7·5	6·5	6·0	6·6

The retarding effect at 48°C. was probably due to the commencement of heat inactivation of the enzyme.

Effect of Acidity.—Small increases of acidity hasten rennet coagulation, and it is probable that both stages are stimulated by this factor. Alkalinity is unfavourable to rennet activity. The general effects of small increases of acidity are to be seen from the following figures of Davis.[2]

Lactic acid increase . .	0%	0·01%	0·025%	0·05%
Time of coagulation . .	22·5	12·0	5·25	2·33

With regard to stage 2 of the change, small increases of acidity will bring more colloidal calcium into solution, and in all probability there will be some increase in calcium ion concentration. An observation by Van Slyke[3] to the effect that milk sour enough to curdle does not give a coagulum with rennet appears to be at variance with the view that rennin is most active at its iso-electric point.[4] It will be remembered that casein and rennin possess almost the same iso-electric point, namely *p*H 4·5 and 4·6 respectively. This apparent contradiction is partly explained by changes which occur in the colloidal tri-calcium phosphate. This substance is necessary for the production of the typical gel structure of rennet coagula. It is possible for rennet to attack calcium caseinate solutions free from colloidal calcium phosphate, but the resulting calcium para-caseinate does not show the typical gel structure. The author[5] has shown that as acidity develops in milk, more and more colloidal tri-calcium phosphate is removed from the casein complex until at the point of curdling none

[1] Lind, 1931, *Hansen's Dairy Bull.*, 16, 13.
[2] Davies, 1939, *Milk Trade Gazette*, December.
[3] Van Slyke and Price, 1941, *Cheese*, p. 119.
[4] Davies, 1939, *Chemistry of Milk*, p. 328.
[5] Ling, 1936, *J. Dairy Res.*, 7, 145.

remains. At the same time more and more of the chemically combined calcium of calcium caseinate is dissolved by increasing acidity and at the point of curdling little if any remains. It is not surprising therefore to find that coagulation does not proceed in the normal manner under these very exceptional conditions of acidity.

The relationship between the hydrogen ion concentration of milk and the time of rennet coagulation is illustrated by the following:[1]

*p*H of the sample . . .	5·7	6·08	6·52	6·98	7·53
Time of rennet coagulation in seconds	29	48	196	1186	no coag.

The Balance of Soluble Mineral Matter.—Of the two stages of rennet coagulation it is the second which is most influenced by soluble mineral matter. The sensitivity of this stage to calcium ions has been referred to earlier in this section. It is considered that the retarding effect on rennet coagulation of heating milk is at least partly due to conversion of some soluble calcium to the colloidal condition. This view is based upon the well-known fact that addition of a small amount of a soluble salt like $CaCl_2$ will restore the normal coagulating properties of milk which has been heated. There is need to exercise care in the interpretation of this observation. In the first place, it is by no means certain that the reduction of soluble calcium which is the result of heating is accompanied by a reduction in concentration of calcium ions. It may be so, but no precise information on this point is at present available. Again, it must be realised that addition of calcium chloride may or may not increase the concentration of calcium ions, but it certainly does increase the hydrogen ion concentration.

There are great differences between individual samples of milk in their behaviour towards rennet, and it was only natural to assume that different amounts of calcium contained therein might afford an explanation. In their examination of the mixed milk of ten Shorthorn cows, Golding *et al.*[2] found a negative correlation between rennot action and the percentage of total calcium in the milk. The author,[3] examining the mixed milk of the College herd

[1] Davis, 1941, *Symposium on Cheese*, Soc. Chem. Ind., 7.
[2] Golding *et al.*, 1935, *J. Dairy Res.*, 6, 6. [3] Ling, 1937, *J. Dairy Res.*, 8 173.

attempted to find the relationship between the time of rennet coagulation and the percentage of *soluble* calcium. *Contrary to expectation, as the latter increased the former increased also.* The ratio soluble inorganic P_2O_5 to soluble CaO showed a positive correlation with rennet action, a high ratio being associated with more rapid coagulation and vice versa. These results suggested that the salt balance known to influence the heat coagulation of casein might also apply to rennet action. Citric acid is known to have a strong de-ionising effect upon soluble calcium, and if a large proportion of soluble calcium in a sample of milk exists in the form of calcium citrate the time of coagulation may be increased on account of a low concentration of calcium ions. The following table shows that as the percentage of soluble calcium citrate (calculated) increases rennet coagulation is progressively retarded.

Time of coagulation in minutes	% Soluble CaO	% Soluble inorganic P_2O_5	Ratio of sol. inorganic P_2O_5 to sol. CaO	Calculated % soluble calcium citrate. Expressed as CaO
1·6–1·8	0·059	0·089	1·506	0·028
1·8–2·0	0·058	0·089	1·557	0·032
2·0–2·2	0·062	0·085	1·414	0·037
2·2–2·4	0·063	0·084	1·340	0·038
2·4–2·6	0·059	0·086	1·446	0·035
2·6–2·8	0·064	0·084	1·320	0·039
2·8–3·0	—	—	—	—
3·0–3·2	—	—	—	—
3·2–3·4	0·068	0·081	1·206	0·056

The matter cannot be dealt with satisfactorily until more is known of the ionic equilibria in milk, but in the meantime it is clear that the presence of high or low percentages of calcium in milk cannot justify the assumption that the milk will coagulate quickly in the former case or slowly in the latter.

Previous treatment of the milk as affecting rennet coagulation.—The reasons for the retarding effect of heat are still obscure. The well-known observation that addition of soluble calcium salts to heated milk restores the normal clotting rate has led to the *assumption* that changes in soluble calcium are responsible. As shown in the previous section, the addition of a calcium salt to milk has many secondary effects. While it is *probable* that changes in the normal

calcium equilibrium are concerned, the matter cannot be considered proven.

Of the known effects of heat on milk the following are likely to affect rennet coagulation:

(*a*) changes in the calcium equilibrium

$$\leftarrow \leftarrow \text{ ionic} \rightleftarrows \text{molecular} \rightleftarrows \text{colloidal}$$

(*b*) alterations in the physical condition of casein

(*c*) denaturation of albumin and globulin and their deposition on the casein particles.

Other factors there may be which have yet to be discovered and the student is strongly urged to keep a very open mind on the subject.

Davis[1] records that the time of coagulation increases up to 6 hours from the time of milking, and considers that this effect is in part due to loss of carbon dioxide gas. The author[2] found that evening milks gave a longer coagulation time than morning samples, the mean values being 2·45 mins. and 2·00 mins. respectively. As there were no differences in composition which could be held responsible for these differences, it was considered likely that the longer period of storage at 45°F. in the case of the evening samples might be a contributing factor. The morning samples were stored for 3–4 hours, whereas the evening ones were kept for 15 to 16 hours at that temperature. To check this supposition, rennet tests were conducted on samples of mixed milk kept in cold store at 45°F. for various lengths of time. The results are shown in the table:

Sample	A		B		C		D	
Age in hours . . .	5	12	3	27	3	10	3	27
Time of coag. in minutes .	4·3	5·0	3·4	3·7	2·8	3·1	1·9	2·4

Sample	E			F			G	
Age in hours . . .	3	10	27	4	10	28	3	27
Time of coag. in minutes .	2·1	2·4	2·4	2·5	2·8	2·9	3·3	3·6

Storage at 45°F. certainly prolongs the time of coagulation, but it is not yet possible to offer an adequate explanation.

[1] Davis, 1939, *Milk Trade Gazette*, December. [2] Ling, 1937, *J. Diary Res.*, 8, 173.

Inhibitory Substances—Rennet action may be inhibited by adsorption of higher fatty acids such as lauric acid on the casein particles. Alkalis and salts of the alkali metals are also unfavourable. The soluble proteins albumin and globulin are known to have a retarding effect, and this is one reason for the difficult coagulation of mastitis milk. Moir[1] found that the whey obtained from cheesemaking with heated milk contained less nitrogen than that obtained from unheated samples. He suggested that some deposition of denatured albumin and globulin occurs on the casein particles and that this was one reason for the slower coagulation of heated milks.

The Physical Nature of Rennet Curd.—The curd produced by rennet may vary in physical properties between a hard rubbery texture at one extreme and a loose soft type at the other. In general it may be said that conditions which favour rapid coagulation give the hard rubbery type of curd whereas prolonged coagulation favours the softer curds. Apart from this, however, there are great differences between the milks of individual cows of any one breed or sometimes amongst individuals of the same herd. This subject will receive further consideration in a later section. At 15°C. (60°F.) the curd is soft, spongy, and loosely bound, while between 24–45°C. (77–113°F.) the curd is much firmer, and within this range its elasticity increases with increasing temperature of coagulation. At temperatures above 50°C. (122°F.) the curd becomes soft and loose. Small increases of acidity tend to give firmer coagula but larger ones, by their effect on colloidal tri-calcium phosphate may result in the formation of flaky curds. Reference has been made to the fact that addition of small amounts of calcium chloride is able to restore the normal rate of coagulation of a heated milk. It has another advantage in improving the texture of the curd which would otherwise be softer than normal. Increases of soluble calcium favour harder curds, but here again it is probably a matter of calcium ions rather than soluble calcium. Milk of low casein content coagulates slowly and tends to have a soft curd. Dilution with water tends to produce the same effect.

In making hard cheese, the milk must develop acidity during the various stages of the process, because, as will be seen later, the physical character of the finished cheese and its moisture content depend so largely upon the correct development of acidity at each

[1] Moir, *J. Dairy Res.*, 1930, 1, 149 ; ibid., 1931, 2, 68 ; ibid., 1932, 3, 80.

stage of the process. For obvious reasons it is better to add a suitable starter which is able to produce lactic acid with no off-flavours than to rely upon the natural micro-organic population of the milk. Addition of starter and the attainment of the correct acidity for renneting are very necessary preliminaries for hard cheesemaking. The making of soft cheese is very different in so far as the lactic acid development occurs *after* the curd has been produced.

The "gel" which is first formed by rennet action, gradually assumes some very interesting properties. If the surface is broken by gentle pulling with the finger, whey will exude until, in some obscure manner, a new surface is formed. The gel is a network of fibres of the calcium para-caseinate–tri-calcium phosphate complex in which fat globules and whey are trapped, and it would appear that the protein and fat are able to form a surface which to some extent encloses the whey. When this surface is broken it is able to reform. This has an important bearing on subsequent operations.

In making hard cheese the moisture content of this "gel" must be reduced from about 87 per cent to approximately 40 per cent at milling, and in this period the texture of the curd must assume that degree of resilience so familiar to the experienced cheesemaker. Davis[1] has studied the manner in which whey exudes from the curd under the influence of cutting, acidity development, and scalding. All three are combined in practice, but it is interesting to find that cutting has the greatest single effect.

As the whey exudes from the curd under the combined influence of acidity and increase of temperature in the scalding process, it shrinks and gradually assumes a tougher, more elastic texture. It is believed that lactic acid enters into some form of chemical union with the curd, but there is no direct evidence in support of this belief. A critical stage in the making of hard cheese is reached when a decision has to be made regarding the attainment of correct texture of the curd before 'pitching.' Normally time, temperature, acidity observations are of great assistance when combined with the cheesemaker's keen observation on the 'feel' of the curd. An attempt has been made by Scott Blair and his associates[2] to put the matter on a scientific basis by application of the science of Rheology (i.e. deformation and flow of matter).

[1] Davis, 1941, *Symposium on Cheese*, Soc. Chem. Ind., p. 7.
[2] Scott Blair *et al.*, 1940, *J. Dairy Res.*, 11. 187; 1941, ibid., 12. 170; 1942, ibid., 13, 73.

Their method is based on the behaviour of the curd when gathered into a wire mesh cylinder, from which it is removed after a period of drainage, by inverting on a plate and careful withdrawal of the cylinder. In the early stages of the scalding process the curd merely slumps outwards, but as its texture improves it behaves more like a sponge. From this, the whey drains away quite freely, and its place being taken by air, the curd assumes a firm spongy consistency. If it were possible to withdraw equal weights of curd, the height of this cylindrical sponge would increase as the texture improves. As it is difficult to take equal weights of curd, the weight removed is recorded on a spring balance after waiting a definite length of time for drainage, and the height of the cast cylinder of curd is divided into this weight to give the pitching number. The reader is advised to consult the original papers for practical details of the test. Until the method has been tested under practical conditions it is impossible to assess its value, and some time must elapse before suitable pitching numbers can be recommended for different types of hard cheese.

In the making of hard cheese acidity develops steadily from the time of adding starter to milling, pressing, and even in ripening; because although whey is run off the curd retains a considerable amount, and the starter organisms are thus able to continue producing lactic acid from lactose within the curd itself. As acidity so largely dominates curd texture, etc., it has become a valuable guide in the various operations of cheesemaking. At the time of writing it is determined by titration of milk or whey with a standard solution of sodium hydroxide (commonly N/9 strength) using phenolphthalein as indicator. The following figures illustrate the changes of acidity at various stages in the making of Cheddar cheese: Milk brought to an acidity of 0·19–0·23 per cent for renneting, acidity of the whey at subsequent stages being: 0·14–0·15 per cent after cutting the curd, 0·18–0·19 per cent at pitching, 0·24–0·28 per cent from piled curd, 0·65–0·95 per cent in whey from curd before grinding, and the whey from the press 0·95–1·10 per cent. The fall in acidity from 0·19–0·23 to 0·14–0·15 is due to removal of casein which is sufficiently acid to combine with the sodium hydroxide solution used in the acidity test.

The limitations of the acidity test have been discussed fully in Chapter II, but in spite of its many shortcomings, it is able to

provide useful guidance throughout the operations of cheese-making, especially when combined with keen observation of other characteristics. It is interesting to note how the control of cheese-making has been raised from indirect empirical observations prior to 1899, when Lloyd[1] first introduced the acidity tests, to the present day when rheological measurements are being considered and when serious attention is being given to the desirability of using *p*H measurements either instead of or together with the acidity tests. Hydrogen ion concentration may be determined accurately only by the use of a potentiometer, but it is extremely doubtful whether the normal cheese factory would be justified in aiming at this degree of accuracy. It is by no means established as yet that under the guidance of *p*H values rather than acidity tests, the quality of the finished cheese is improved. The *p*H may be determined approximately using a colorimetric method,[2] but even this is not so rapid or convenient as the usual titration value.

Natural acidity has been discussed in Chapter II when it was seen that some variation may be expected from cow to cow. When milk is bulked at a cheesemaking factory it is unlikely that serious difficulty will arise on this score, but in the making of farmhouse cheddar, M. C. Taylor[3] has observed that when the cheesemaker follows rigidly the rule of adding rennet at an acidity of 0·22 per cent, difficulties arise quite frequently. In her experience, milk which started with a low natural acidity should be renneted before 0·22 per cent according to the rate of acid development, while milks which had a high natural acidity require an acidity greater than 0·22 per cent before rennet is added. To overcome the disturbing influence of variations in natural acidity in cheesemaking on a small scale, the rate of acidity development should be the guide rather than the actual acidity figures. It has been observed that milk of high natural acidity shows no quicker coagulation with rennet than do those of lower values.[4]

An interesting fact about the protein of the newly made cheese is its solubility in warm salt solutions 3–10 per cent strength. It is considered that apart from other considerations the presence of salt in cheese is desirable on this account. With a hard cheese of 33 per cent moisture, the salt percentage on this basis alone should lie

[1] Lloyd, 1899, *Report on Cheese Making*, H.M. Stationery Office.
[2] Davis and Thiel, 1940, *J. Dairy Res.*, 11, 71.
[3] M. C. Taylor, 1929, *Agricultural Progress*, 6, 80.
[4] Rice and Markley, 1924, *J. Dairy Sci.*, 7, 468.

between 1 and 3 per cent. It is considered that 1·66 per cent of salt in New Zealand cheddar cheese is a very desirable concentration.[1] Addition of salt has other effects. It enables freer drainage of whey and checks bacterial and enzyme action. Too much will severely check the normal process of ripening besides being unpleasant to the taste.

The Ripening of Cheese.—Four important changes take place during the ripening process:

1. Enzymatic digestion of protein.
2. Conversion of lactose to lactic acid and other compounds.
3. Loss of moisture by evaporation.
4. Production of flavours.

Protein Digestion.—At the commencement of ripening only about 4 per cent of the total protein is soluble in water, but this proportion increases in a well ripened hard cheese to about one-third of the total or in ripened soft cheese it may rise to two-thirds or even higher. The change may be represented as following a course similar to that which occurs in the digestive tracts of animals, namely:

Insoluble protein→proteose→peptones→polypeptides→amino acids→ammonia.

The complex protein molecules are broken down by successive stages into compounds which become progressively simpler in chemical and physical structure. This process is not merely one whereby an insoluble compound is rendered soluble, for if so, it need only proceed to the *first* stage. In a ripened cheese, representatives of *all* these stages will be found. Other conditions being equal the more moisture the green cheese contains the more rapid and extensive will be the degradation of the protein. These changes are brought about by proteolytic enzymes of the rennet and enzymes secreted by lactic acid bacteria, and, in the ripening of soft cheeses moulds play a very conspicuous part. The following table[2] shows that the rate of ripening in hard pressed cheese depends upon the temperature of the ripening room and is more rapid during the earlier stages of the process, the figures in the 3rd, 4th, and 5th columns being the ratio

$$\frac{\text{Water soluble nitrogen}}{\text{Total nitrogen}} \times 100.$$

[1] MacDowell and Whelan, 1938, *J. Dairy Res.*, 9, 338.
[2] Van Slyke and Price, *Cheese*, 1941, p. 289.

Temperature—	Age of Cheese		
	1·5 months	3 months	6 months
32°F. . . .	12·8	18·64	23·06
55°F. . . .	20·56	31·46	36·09
60°F. . . .	23·14	33·69	39·97
70°F. . . .	29·24	40·13	45·50

Van Slyke and Price also record that by coating cheese with a layer of paraffin wax, losses of moisture are reduced to a minimum and moreover the ripening process is accelerated thereby. Since smaller cheeses offer a greater relative surface area, it follows that they tend to lose moisture more rapidly and the rate of ripening is slower than that of larger ones.

Ripening is also quickened by increasing the quantity of rennet and although its precise mode of action is obscure it is generally agreed that rennet plays some part in the protein degradation during ripening.

Using the ratio soluble to total nitrogen as an index of ripening Van Slyke and Price[1] observed the retarding influence of salt on the process. The following table is compiled from their data:

lbs. salt per 1000 lbs. milk	Age of Cheese					
	1·5 months		3 months		6 months	
	$\frac{\text{sol. N}}{\text{total N}} \times 100$	% salt in cheese	$\frac{\text{sol. N}}{\text{total N}} \times 100$	% salt in cheese	$\frac{\text{sol. N}}{\text{total N}} \times 100$	% salt in cheese
0	23·42	0	34·26	0	40·52	0
1·5	21·80	0·59	32·10	0·70	37·67	0·84
2·5	21·67	0·82	29·92	1·20	34·73	1·15
5·0	18·84	1·29	27·70	1·50	31·70	1·62

Lactose undergoes vigorous fermentation to lactic acid, but as lactic fermentation seldom produces lactic acid alone it is not surprising to find that volatile acids of the acetic, propionic type are also produced.

Evaporation of Water.—Loss of water by evaporation is inevitable, and to some extent desirable, with hard cheese, and the problem of the cheesemaker is to bring these losses under control. The temperature of the ripening room and the humidity of the

[1] Van Slyke and Price, 1941, *Cheese*, p. 292.

atmosphere determine how much evaporation can proceed from the cheese surface. Because higher temperatures are usually accompanied by less humidity it has been observed that more water is lost in six months from hard cheese ripened at 70°F. than at 55°F.[1] The losses per 100 lbs. cheese were 11·1 lbs. and 8·1 lbs. respectively. Comparing the behaviour of two similar cheeses, one kept at 60°F. in an atmosphere showing a relative humidity of 75–80 per cent, and the other kept at the same temperature in a completely saturated atmosphere, Van Slyke and Price found that in six months the former lost 4 lbs. per 100 lbs. cheese whereas the latter actually gained weight to the extent of 0·11 lb. per 100 lbs. Coating the cheese with paraffin wax reduces these losses to a minimum.

It has been recommended that at the early stages of ripening the atmosphere of the ripening room should be 85–90 per cent saturated with moisture and that an appropriate degree of humidity for storage would be 90–95 per cent. If it is intended to measure the relative humidity of the atmosphere with wet and dry bulb thermometers, it is necessary that the thermometers should be able to register small differences of temperature. For instance at 13°C. an atmosphere having a relative humidity of 90 per cent would give a difference of 0·8°C. between the dry and wet bulb readings. If the thermometers are misread and a difference of 0·6°C. is obtained, the relative humidity would be returned as 93 per cent. Unless the thermometers are graduated in fractions of degrees the instrument becomes merely an undecorative piece of furniture. If the factory is not sufficiently large to justify the installation of an air-conditioning plant, the maintenance of correct humidity must remain a matter of trial and error. By swilling floors with water or by hanging damp cloths in the room the humidity may be increased. To reduce humidity it is necessary either to increase the temperature of the room or to improve ventilation. Unfortunately there has been very little work on this subject of humidity control and measurement as related to cheese ripening, except that of Golding who describes a design of ripening-room to maintain a humidity of 88 per cent.[2]

The moist conditions are favourable for the growth of moulds on the surface of ripening cheeses. Of recent years this undesirable

[1] Van Slyke and Price, 1941, *Cheese*, pp. 275, 276.
[2] Golding, 1931, *J. Dairy Res.*, 3, 101.

growth has been checked by dipping the cheese or painting it with a solution of propionic acid or of calcium propionate. The customary strength is in the region of 5 per cent.[1]

Vernon[2] observed that a *controlled humidity* of 80–85 per cent in the ripening-room was effective in reducing shrinkage losses and in the avoidance of mould growth.

The Development of Flavours.—From the foregoing considerations it is clear that the most extensive changes in ripening are concerned with cheese protein, but it is extremely doubtful whether any of the products of proteolysis are themselves responsible for the typical flavours of ripened cheese. In this connection there has been a general belief that pepsin may produce peptones which are the cause of bitter flavours in the ripened cheese but direct evidence is difficult to obtain. However, while it may not be possible to identify cheese flavours with any direct degradation products of the proteins it is certain that the latter changes must precede the former. It is likely that secondary changes in some of these products of proteolysis may contribute to flavour production. During cheese ripening, volatile acids such as acetic, propionic, butyric, caproic, capric, and caprylic may appear as a result of bacterial and enzyme action on lactic acid, lactates, citrates, or some of the products of proteolysis. These, their esters, or their ketones, are *believed* to be responsible for the typical flavours of ripened cheese. Most of these acids are typical fatty acids, and although fat is not appreciably altered in the ripening process the possibility of slight lipolysis occurring should not be overlooked. With hard cheese lipolysis is not appreciable, but with the semi-hard blue-veined type it occurs to a much greater extent through the activity of moulds. The peculiar peppery flavour of Roquefort cheese is associated with caproic, caprylic, and capric acids or their esters. Hiscox *et al.*[3] have introduced improved methods of estimating the volatile acids of ripened cheese which are able to discriminate between those which are dissolved in the cheese fat and those which occur in the water phase. The former are assumed to be the result of fat hydrolysis while the latter are not. With ripened Cheddar cheese the water soluble fraction predominates. Nevertheless, fat soluble acids are present and the connection between this limited hydrolysis and the development of flavour cannot be

[1] C. K. Johns, 1941. *Canad. Dairy and Ice Cream J.*, 20, 36.
[2] T. R. Vernon, 1940. *Dept. Sci. Ind. Res. N.Z.*, p. 10.
[3] Hiscox, Harrison, and Wolf, 1938, *J. Dairy Res.*, 9, 227.

ignored. Lipolysis was observed by these workers to be much more marked in Stilton cheese, and even more so in Roquefort cheese in which mould growth is much more prolonged. An interesting observation was made during the course of these experiments[1] concerning the effect of pricking Stilton cheese to encourage mould growth. The vigorous mould growth following the pricking caused a reduction in water soluble volatile acids and a pronounced increase in the fat soluble fraction.

Davis[2] has examined the action of lipase in the ripening of Cheddar cheese, and has concluded that it makes a significant contribution to flavour. This view finds some measure of support from the fact that pasteurisation of milk for cheesemaking produces a very slow ripening cheese. Addition of 0·025 gm. lipase per gallon of milk produced a very strong flavoured cheese with a peppery odour. Whether it is possible to improve the Cheddar flavour by smaller additions of lipase is at present an open question.

Although it is not yet possible to state which particular compounds are responsible for cheese flavours, it would appear that further exploration along these lines will yield much desired information.

The Composition of Cheese.—At the time of writing there are no standards of composition, although it seemed likely immediately prior to the outbreak of the second world war that the following

Cheese	Water		Fat		Fat in dry matter		% Protein ash and lactic acid	No. of samples
	%	S.D.	%	S.D.	%	S.D.		
Wensleydale .	45·03	3·72	27·72	2·62	50·23	2·42	27·25	111
Caerphilly .	44·67	2·96	28·43	2·00	51·23	2·36	26·90	234
Lancashire .	44·28	1·82	27·39	1·49	49·10	1·88	28·33	403
White Stilton	41·76	4·13	32·44	3·53	55·37	3·14	25·80	124
Gloucester .	41·0	—	28·8	—	49·0	—	30·2	3
Cheshire .	40·53	2·36	29·96	1·83	50·30	2·10	29·51	606
Derby . .	39·95	1·62	30·59	1·62	50·70	2·12	29·46	11
Leicester .	39·79	2·61	30·21	1·43	50.00	1·89	30·00	24
Blue Stilton .	37·64	3·96	35·71	3·52	57·20	3·08	26·65	212
Cheddar .	36·69	2·34	31·82	1·65	50·20	1·98	31·49	363
Cream . .	24·5	3·56	71·6	3·49	—	—	3·9	49

S.D.=Standard deviation of mean.

[1] Hiscox *et al.*, 1941, *Symposium on Cheese*, Soc. Chem. Ind.
[2] Davis, ibid.

recommendations would be brought into effect—a minimum of 45 per cent fat on a dry matter basis; 50 per cent for extra selected blue Stilton cheese; 60 per cent fat minimum for cream cheese. A very comprehensive examination of the water–fat relationship of many types of cheese has been made by Nicholls[1] from whose results the table on p. 161 has been prepared.

The following analyses of Lindet[2] also illustrate the effect of ripening on cheese proteins:

	% Water	% Fat	% Protein	% Ammonia	$\frac{\text{sol. N}}{\text{total N}} \times 100$
Coulommiers (double cream)	57·8	25·0	13·0	0·13	44·4
Camembert	53·8	22·0	17·1	0·23	86·1
Brie	53·5	22·5	18·0	0·18	58·1
Pont l'evêque	51·0	23·1	17·8	0·13	43·9
Gruyère	35·7	28·0	28·9	0·05	22·9
Cheshire	31·1	32·3	30·9	0·20	30·1

Acid Curd Cheese.—This type of cheese in which the curd is formed by the action of developed lactic acid on casein, requires very little ripening as the flavours are derived solely from lactic fermentation. Apart from the difference in method of formation, the curd differs from rennet curd in possessing very little mineral matter.

Adulteration of Cheese.—The substitution of skimmed milk for whole milk or addition of the former to cheese milk is a form of adulteration which can be detected by direct analysis. The percentage of fat in the dry matter will be below 45 per cent, and the ratio of fat to protein will be abnormally low. It is possible to emulsify margarine fat into separated milk and to manufacture from the mixture 'margarine cheese.' This may be detected by extracting the fat from cheese and examining its chemical characteristics, i.e. soluble and insoluble volatile fatty acids, iodine value, etc.

Processed Cheese.—Crustless, or processed cheese has become so popular of late years that some mention of its preparation is considered appropriate to this section. Cheeses are graded into groups according to age, flavour, texture, the bandages

[1] Nicholls, 1941, *Analyst*, 66, 265.
[2] Lindet, quoted by Houdet, 1910, *Laiterie, Beurrerie, Fromagerie*, p. 125.

removed, and the cheeses trimmed to remove rinds, etc. They are then ground and blended in the correct proportions, after which the mixture is heated, usually in steam-jacketed kettles, to a temperature of 150°F., at which they are held for 3 to 10 minutes. Fat separates at first but is reabsorbed if the blend does not contain too high a proportion of very young or old cheeses. When sufficiently scalded, the cheese can be drawn out into long fibres. Although some cheese requires no emulsifying agent, substances like Rochelle salt (sodium potassium tartrate), sodium citrate, or di-sodium phosphate are commonly used for this purpose. The latter if present in amounts greater than 2 per cent tends to darken the tinfoil in which the finished product is wrapped. Of the three substances mentioned sodium citrate appears to be most satisfactory when used up to 3 per cent concentration. One factor, the control of which causes some difficulty, is loss of moisture during heating. Attempts are made to compensate this loss by addition of water before steaming, or if emulsifiers are used they are dissolved in enough water to compensate losses by evaporation.

It is desirable that special consideration should be given to processed cheese when the time is opportune to establish standards of composition. It would seem that a minimum of 45 per cent fat on a dry matter basis and a maximum water content of 42 per cent would be reasonable stipulations.

Metallic Contamination of Cheese.—When cheese is wrapped in metal-foil there is always a tendency for small amounts of the metal to pass into the cheese, the extent of such contamination depending on the age and acidity of the sample and the purity of the foil. Pure tinfoil and surfaced foils in which a thin film of inert material separates the cheese from the metal, have been found most satisfactory. Templeton and Sommer[1] examined the properties of various foils, and found that a commercial tinfoil containing 97 per cent tin and 3 per cent antimony gave satisfactory results. Discoloration was likely to occur if a phosphate emulsifier was used, especially if the cheese is less acid than indicated by a *p*H 5·8. Thin coatings of shellacs and resins on aluminium foil were also found useful as wrapping foils, although this metal does not cling to the cheese like tinfoil: a matter of some importance since it is necessary that the foil should seal the cheese.

[1] Templeton and Sommer, 1937, *J. Dairy Sci.*, 20, 231.

In a previous chapter reference was made to the apparent immunity of cheese fat to oxidative changes stimulated by copper. Davies[1] examined a number of cheeses and found their copper content as follows:

Camembert 6 parts per million, Petit Gruyère 9·2 p.p.m., Emmenthal 18·1 p.p.m., all having been made in copper vats, while other cheese ranged from 0·6–2·0 p.p.m. Although conditions of oxygen tension did not permit fat oxidation, he observed that 36 p.p.m. of copper produced an abnormal flavour and considerably inhibited the ripening process.[2] Barnicoat[3] added various metals to cheese milk prior to the addition of starter and observed the effects of such contamination on the finished cheese at different stages of maturity. Defects of colour and flavour were apparent in matured cheese after 9 months which were not evident 14 days after manufacture. The metals were added to the milk to give concentrations of 3 and 7 parts per million of metal. In all cases most of the metal passed into the cheese curd. Flavour defects were most obvious in cheeses contaminated with copper, iron, aluminium, and lead, and were described as "bitter," "sharp," and "pungent."

Sulphydryl compounds are normal products of protein degradation in the ripening process, and it was found that copper and to a lesser degree iron and zinc, exerted an appreciable retarding action on the production of these substances. Barnicoat found that in the concentrations used, these metals did not affect the time of rennet coagulation.

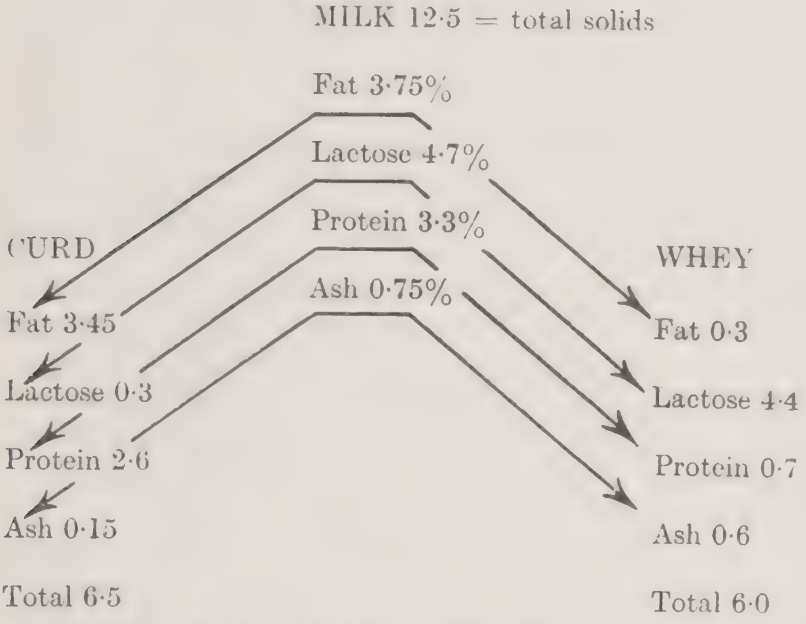
The lead-contaminated cheeses showed black discoloration due to the formation of lead sulphide by reaction with the sulphydryl compounds. Some black haloes in cheese were attributed by Davies[4] to fragments of lead-containing solder scraped from the vat by cheese knives. Again lead sulphide was the cause of the discoloration. Leitch[5] records a rare instance of lead contamination caused by the use of annatto which had been adulterated with red lead. In this case the dark discoloration was diffused throughout the cheese.

While it is natural to assume that the dark muddy discoloration observed in copper or iron-contaminated cheese is also due to union of these metals with sulphydryl compounds to form the respective metallic sulphides, the observations of Barnicoat do

[1] Davies, 1931, *J. Dairy Res.*, 3, 86. [2] Davies *et al.*, 1934, *J. Dairy Res.*, 5, 144.
[3] Barnicoat, 1937, *J. Dairy Res.*, 8, 53. [4] Davies, 1932, *Analyst*, 57, 95.
[5] Leitch, 1929, *Scott. J. Agric.*, 12, 308.

not support this view. The experimental evidence led to the conclusion that these two metals unite with protein to give practically colourless proteinates in cheese, which on exposure to air suffered oxidation into darker coloured compounds.

The distribution of the principal milk constituents between cheese and whey is illustrated as follows:



It is clear that whey removes a large proportion of lactose and mineral matter originally present in milk. It will also remove a very considerable proportion of the water-soluble vitamins, and it is therefore highly desirable that these losses should be compensated as far as possible by conversion of whey into human feeding-stuffs. Normally some 60 per cent of milk calcium and 57 per cent of milk phosphorus are retained by the cheese. Most of the mineral losses occur before the whey is run.[1] If the whey is too acid at running, the mineral losses are much more severe, and the resulting cheese is on this account more acid.[2] Mattick[3] has examined the mineral content of various cheeses, some of her results being as shown in table on p. 166.

The similarity between the hard pressed cheeses is very striking. Mattick also observes that most of the mineral losses occur up to

[1] McDowall and Dolby, 1935, *J. Dairy Res.*, 6, 218.
[2] Dolby, McDowall, and McDowell, 1937, *J. Dairy Res.*, 8, 74.
[3] Mattick, 1938, *J. Dairy Res.*, 9, 233.

	Cheddar	Cheshire	Leicester	Lancashire	Stilton
Ash% . .	3·75	3·59	3·86	3·93	2·82
Ca% . .	0·80	0·69	0·76	0·66	0·107
P% . .	0·51	0·47	0·49	0·44	0·268
Ratio Ca/P . .	1·57	1·47	1·55	1·50	0·41
Ca retained . .	65·5%	65·4%	68·0%	65·8%	7·8%
P retained . .	58·1%	51·2%	55·0%	60·0%	21·98%

the time of running the whey. The greater losses incurred in the making of Stilton cheese are very significant, and are borne more by calcium than phosphorus. White specks which appear occasionally in mature Cheddar have been identified as calcium lactate.[1] Others of similar appearance have been shown to consist of the amino acid tyrosine, a product of ripening.[2]

The losses of vitamins B_1 and B_2 in the whey are respectively 90 per cent and 75 per cent of the total. Assuming these two vitamins to be confined to the water phase, much greater losses would be anticipated, from which it is apparent that some proportion of them is normally combined with milk protein. Fat soluble vitamins A, D, and E are retained in the fat.

ANNATTO.—Annatto, the colouring matter used for butter and cheese contains a red-yellow pigment bixin, which is named after the shrub *Bixa orellana* which bears seeds from which the pigment is extracted. Bixin is a carotenoid, having the formula

$$CH_3{\cdot}OOC(CH=CH-\overset{CH_3}{\overset{|}{C}}=CH)_2CH=CH(CH=\overset{CH_3}{\overset{|}{C}}-CH=CH)_2COOH.$$

The potassium salt of the acid is the basis of cheese annatto, which is essentially an alkaline solution of this salt. In cheese this coloured salt is bound by the protein. For colouring butter, the pigment bixin is dissolved in oil, from which it passes into butterfat. Barnicoat[3] has examined cheese annatto and finds that the pink constituent becomes more apparent as acidity increases between *p*H 6 and 4·8. As the formula indicates, the compound is very unsaturated and might be the centre of oxidation-reduction changes. Barnicoat observed that bleached or pink discoloration was sometimes caused by oxidation of annatto.

[1] McDowall and McDowell, 1939, *J. Dairy Res.*, 10, 118.
[2] Dorn and Dahlberg, 1942, *J. Dairy Sci.*, 25, 31.
[3] Barnicoat, 1937, *J. Dairy Res.*, 8, 61.

Bleaching was generally observed in the cracks of cheeses examined. When the fat of milk was replaced by butterfat which had oxidised to the extent of a tallowy taint, cheese made therefrom showed extensive bleaching of annatto. Sulphydryl compounds were considered to act as catalysts in these changes.

The colour of annatto solutions was found to be subject to a slow fading, especially under the influence of light or in the presence of traces of copper. Barnicoat suggested that the solutions would keep better in glass containers than in metal drums, but Carrie[1] found that the loss of colour of such solutions kept in steel drums over a period of three years amounted to 10 per cent only.

Soft Curd Milk.—The nature of rennet curd obtained from individual cows shows great variation in properties, sometimes being loosely knit and soft, while at others it is much firmer and elastic. Even milk from one cow will show considerable variation in this respect. Mastitis is known to make cheesemaking difficult through its retarding action on rennet and the production of a very loose type of curd, but quite apart from this cause, there have been instances when individual cows whose milk is normally suitable for cheesemaking, have suddenly produced milk which is unfavourable to rennet action.

In order to obtain a measurable index of the texture of curd, Hill[2] introduced a method whereby milk was coagulated by pepsin and calcium chloride solution, and the curd tension measured as the resistance in grammes to the cutting action of a specially designed curd knife. Values obtained by this method varied between 50–90 grammes, values less than 30 indicating soft curd. The test has been modified in several directions but the principle remains the same. It was designed to differentiate between milks giving soft curds suitable for infant feeding, and those giving the harder type which were therefore more suitable for cheesemaking. More recently Rowland and Soulides[3] have designed a method whereby the firmness of rennet curd may be measured and used as an index of the suitability of milk for cheesemaking. Their method is very different from that of Hill and depends on observations of the extent of deformation of the curd when a small weighted disc is placed on its surface. With this instrument they were able to measure curd firmness, and it is

[1] Carrie, 1938, *J. Dairy Res.*, 9, 72. [2] Hill, 1923, *J. Dairy Sci.*, 6, 509.
[3] Rowland and Soulides, 1942, *J. Dairy Res.*, 13, 85.

hoped that it may be possible to correlate this property with the chemical composition of milk. Both Hill and Rowland found very considerable variation in these two curd properties.

The question of the production and distribution of soft curd milk for infant feeding has received much attention in the United States of America. As the human infant sometimes experiences difficulty in digesting cow's milk, even when modified to the same composition as human milk, it seemed at first that it might be possible to segregate those cows known to produce soft curd milk, and to built up herds from them for the sole object of supplying infants and hospitals with this commodity. This was not practicable, for only about 1 per cent of the cows examined gave soft curd milk, and although they were of more frequent occurrence in the Friesian and Ayrshire breeds, there was very considerable variation not only between different individuals but in the same cow at different times. If the cause of soft curd milk were known, it might be possible to modify hard curd milk by appropriate measures in order to render it suitable for infant feeding. Attempts to correlate softness of curd with chemical and physical properties of milk have not met with any signal success. It may well be that Cherbuliez and Jeannerat are correct in their view that casein of cow's milk is a mixture (see page 37), and that curd softness is due to unusually high proportions of the constituent which is not affected by rennet.

The curd tension may be reduced by dilution with water, heating, addition of lime water, homogenisation, and intense sonic vibration, but either the effects are not great enough to soften the curd as desired or the necessary equipment would render the method impracticable. Two methods have met with outstanding success. One is the passing of milk through a zeolite filter such as is used for removing the hardness of water. Soluble calcium is thus replaced by sodium according to the base exchange principle:

$$\text{Ca-Milk} + \text{Na-Zeolite} = \text{Na-Milk} + \text{Ca-Zeolite}$$

Curd tension may be reduced to zero by this means, but the great objection lies in the removal of soluble calcium which is so necessary to the growing infant. The second method depends upon the calcium ion depression brought about by addition of sodium hexa-metaphosphate, and the consequent softening of the curd. The following figures[1] illustrate this effect:

[1] Schwartz *et al.*, 1940, *J. Dairy Sci.* .23, 19.

mg. sodium hexa-meta-phosphate per 100 ml. of milk . . .	0	35	40	45	50	slightly more than 50
Curd tension in grms. .	55	40	30	20	0	no curd

Addition of 41 mg. of this substance, the trade name for which is Calgon, is considered sufficient to soften 100 ml. of milk. This method has the advantage of being simple to operate and does not remove any nutritive constituent. It remains to be seen whether medical opinion on the suitability of milks modified in this way for infant feeding is as favourable as the preliminary physical tests would indicate. Those interested in the subject should consult Doan's comprehensive review, 1938, *Journal of Dairy Science*, volume 21, p. 739.

CHAPTER VII

CONDENSED MILK AND MILK POWDER

THE condensation and drying of milk represent the modern attempts to conserve the valuable nutritive properties of this food. In making butter, the fat of milk is concentrated and to some extent 'preserved,' while in cheese the fat and protein of milk are concentrated and similarly conserved. The history of the making and utilisation of these two products is indeed ancient. Their originators are unknown, but, wittingly or unwittingly, they achieved signal success in milk conservation by eliminating much of the water which is responsible for its perishable nature. The modern conception of direct concentration of whole milk is but a development of an ancient discovery. In 1298, Marco Polo recorded that the Tartar armies were supplied with a variety of dried milk. In spite of the great antiquity of the principle of water elimination, the large-scale manufacture of condensed and dried milk belongs to the nineteenth century when the vacuum evaporator and other drying machinery became available.

The vacuum evaporator uses the well-known effect of reduction of pressure in lowering the boiling-point of liquids, and was invented by Howard to whom was granted a British patent in 1813. It was first used for condensing milk in 1835. To Borden in America must be given credit for placing the condensed milk industry on a sound economic basis and his process was patented in America and this country in 1856. Milk powder first appeared in Britain in 1855, and its manufacture was to pass through many stages of evolution before the modern processes were established. These two industries are highly technological, and any attempt to cover the whole subject in a book of this type would be futile and very misleading. *It is intended therefore to present fundamental principles rather than a mass of technical details.*

Condensed Milk.—High sugar concentration, with or without elimination of water was a well-tried method of preservation, and it was applied to milk conservation in 1847 when the first sweetened condensed milk was prepared. Although it was later

discovered that addition of sugar was unnecessary provided the condensed milk were sterilised, both processes are in operation to-day. The requirements of the Foods and Drugs Act determine to some extent the procedure adopted in both processes. Immediately prior to the Second World War, the following standards were in force:

	% Fat	% Total milk solids
Sweetened and unsweetened condensed whole milk. . .	9	31
Sweetened condensed skimmed milk	—	26
Unsweetened condensed skimmed milk	—	20

The same regulations require that the containers should be labelled clearly, stating whether sweetened or unsweetened, skimmed or whole milk. If made from skimmed milk the statement "not to be used for babies" must be included. A further statement of the number of pints of reconstituted milk or skimmed milk equivalent to the contents of the container is necessary. In this connection whole milk is considered to contain 3·6 per cent fat and 12·4 per cent total solids, and skimmed milk 9 per cent non-fatty solids. It should be pointed out that unsweetened condensed separated milk rarely appears on the market.

From these standards of composition, the ratio fat: non-fatty milk solids in the condensed whole milks must be 9/22 or 1/2·44, and it is therefore desirable that milk should be standardised to this ratio before condensing. It is also apparent from these requirements that to concentrate milk of 12·4 per cent total solids to give 31 per cent in the finished product requires concentration to $\frac{1}{2{\cdot}5}$ the original bulk. Milk standardisation involves accurate measurement of the weight of milk and equally accurate estimations of its fat and non-fatty solid content. Cream or separated milk are added according to whether fat or non-fatty solids are to be supplemented. For example, suppose 6000 lbs. of milk containing 3·2 per cent fat and 8·9 per cent non-fatty solids are to be condensed, and it is required to calculate how much

cream of 30 per cent fat and 6·4 per cent non-fatty solids must be added to standardise to the correct ratio.

6000 lbs. of milk supply $\frac{6000 \times 3{\cdot}2}{100} = 192$ lbs. fat.

6000 lbs. of milk supply $\frac{6000 \times 8{\cdot}9}{100} = 534$ lbs. non-fatty solids.

The desired ratio is 1 lb. fat to 2·44 lbs. non-fatty solids. i.e. 192 lbs. fat to 468·5 lbs. non-fatty solids.

Surplus of non-fatty solids = 534 — 468·5 = 65·5 lbs.

1 lb. of cream contains 0·3 lb. of fat which will balance 0·732 lb. S.n.F.

1 lb. cream contains 0·064 lb. non-fatty solids, so that each lb. of cream is able to balance 0·732 — 0·064 = 0·668 lbs. non-fatty solids.

65·5 lbs. of surplus non-fatty solids are balanced by addition of $\frac{65{\cdot}5}{0{\cdot}668} = 98$ lbs. of cream.

This quantity of cream added to the 6000 lbs. of milk will standardise to the desired ratio of 1 fat to 2·44 of non-fatty solids. A similar calculation is used to calculate the quantity of separated milk required to balance an excess of fat.

Standardisation is therefore a necessary preliminary to condensation of whole milk, whether sweetened or unsweetened, but thereafter the manufacturing processes are different.

Sweetened Condensed Milk.—After standardisation the milk is forewarmed with the object of reducing the micro-organic population. It further assists the solution of sugar which is subsequently added and enables boiling to take place before any scorching can occur at the surface of the heating coils of the evaporator. Forewarming temperatures near boiling-point render the product more liable to thickening defects on storing, and Hunziker[1] recommends 75–85°C. (167–185°F.).

Sugar may be obtained in a very pure condition, but it must be stored dry in order to avoid contamination with yeasts and moulds which would ferment the finished product. The quantity of sugar to be added is limited at the upper extremity by the saturation level of sugar in water, and at the other extreme by the minimum concentration of sugar which would exert the desired preservative effect. On the basis of 31 per cent total milk solids about 17 lbs. of sugar are added per 100 lbs. of milk. The common practice is to dissolve the measured quantity in the warm milk after fore-warming.

[1] Hunziker, 1935, *Condensed Milk and Milk Powder*, p. 62.

The vacuum pan is sterilised and evacuated to 10 to 6 inches of mercury. Milk is drawn in to cover the heating coils, whereupon the steam is admitted to them, and as the milk boils vigorously the inflow of milk regulated so as to maintain a constant level. Pressure is at first kept high enough to prevent excessive foaming after which it is reduced to about 4 inches. Under these conditions milk boils at 130–145°F., temperatures well below those which bring about the physical and chemical changes mentioned in a previous chapter (page 103). Reference has been made to the importance of using the correct metal for the heating coils (page 113), and to the undesirability of tinned copper for this purpose. Evaporation must proceed until the correct degree of concentration has been attained. Judging the correct moment to stop the process requires a high degree of skill. In this matter, specific gravity tests are a valuable aid, for provided the milk has been standardised accurately, the specific gravity of the condensed product is clearly correlated with its total solid content.

At the correct degree of concentration the contents of the evaporator constitutes a saturated solution of lactose at 130–140°F., and it follows, therefore, that crystals of lactose will separate on cooling. If small enough these crystals will not be detected, but if too large they will impart a gritty texture. Hunziker[1] gives the following dimensions of the crystals and their effect on the smoothness of the product. Less than 10 microns (i.e. 0·01 mm.) smooth texture

10 to 12 microns, good but slightly pasty
12 to 15 microns, mealy
15 to 30 microns, mealy slightly sandy
over 30 microns, objectionably sandy.

The gritty texture becomes apparent after prolonged standing and it would appear that the defect is caused by continued slow growth of the lactose crystals after the condensed milk has cooled to normal temperatures. To avoid this defect it is necessary to create conditions favourable to a maximum crystallisation of lactose so that crystallisation is complete by the time the milk reaches ordinary temperatures. The condensed milk is accordingly cooled rapidly to about 86°F., the temperature most favourable to rapid crystallisation of lactose, and by addition of lactose powder or some of the previous day's condensed milk the

[1] Hunziker, 1935, *Condensed Milk and Milk Powder*, p. 388.

liquid is 'seeded' with lactose crystals. Under these conditions and by constant agitation, crystallisation proceeds satisfactorily, care being taken to control the rate of cooling so that some 50 minutes are taken to reach a temperature of 75°F. The milk is then cooled to 60–63°F., and in some factories the dissolved air is removed by reducing the pressure. Filling into cans and sealing are in most factories carried out by machinery.

Unsweetened Condensed Milk.—The various stages in the manufacture of unsweetened condensed milk may be represented as follows:

1. Inspection of milk as it arrives at the factory.
2. Analysis and standardisation.
3. Forewarming to 95°C (203°F.) for 10 to 15 minutes.
4. Vacuum evaporation.
5. Homogenisation of the evaporated product.
6. Mechanical filling and sealing of cans.
7. Sterilisation at 240°F. for 15 minutes in the cans.
8. Cooling, and incubation to test the quality of the product.

The keeping qualities of this type of condensed milk depend upon the final sterilisation, and owing to the fact that concentration lowers the heat stability of casein, there is always a risk of coagulation of this compound during the operation. The various factors known to lower the heat stability of casein are well known even although they are not well understood. So far as the milk is concerned, developed acidity and an abnormal salt balance are the most commonly occurring. Of these two factors, the former is more common. Platform tests for developed lactic acid are very desirable, but, unfortunately, they are not forthcoming, and as a consequence reliance has to be placed upon such indirect methods as smell, taste, and titratable acidity. The limitations of the latter method have already received attention. The alcohol test[1] and the phosphate test[2] have been used on various occasions, but none seems entirely satisfactory, that is to say, no one test yet devised is able to predict with certainty how the evaporated product is going to behave under the drastic conditions of the final sterilisation.

After inspection and analysis the milk is standardised to the correct ratio of fat to non-fatty solids, and if casein stabilisers are to be added it is desirable that they should be added at this stage.

[1] Sommer and Binney, 1923, *J. Dairy Sci.*, 6, 3.
[2] Ramdsell, Johnson and Evans, 1931, *J. Dairy Sci.*, 14, 93.

Forewarming reduces the micro-organic population and has a valuable stabilising effect on casein. Procedure varies somewhat, but ten to fifteen minutes holding at 95°C. (203°F.) is a common method of forewarming. Evaporation is carried out as with sweetened condensed milk, the hydrometer again proving a valuable instrument for checking the correct degree of condensation. It is particularly important that the milk should not be more concentrated than necessary when sterilised, for it is estimated that with every increase of 1 per cent in non-fatty solids, there is a fall of about 1·3°C. in the temperature of heat coagulation of casein. To arrive at the required concentration some condenseries slightly over concentrate, and add the calculated amount of water.

To prevent fat separation in the finished product, a defect which caused a great deal of trouble in the early days of the industry, the evaporated milk is homogenised at 2,500 to 3,000 lbs. per square inch pressure at 130–145°F. Filling and sealing the cans is for the most part a mechanical operation.

Because of the ever-present tendency of casein to coagulate during sterilisation it is clear that very careful control must be exercised throughout this process. Hunziker's[1] comments are emphatic: "No formula can be laid down which can be depended upon to give uniformly satisfatory results under all conditions. Nor does the proper sterilisation depend on one particular formula. There are numerous ratios of temperature, time of exposure, and extent of agitation which, when adjusted to local conditions, may give satisfactory results." A common procedure is as follows: The tins are raised to 240°F. in 15–20 minutes, with constant agitation to prevent the milk from cooking to the tin. Agitation is stopped while they are held at 240–245°F. for no less than 15 minutes. They are then cooled with cold water to bring the temperature down to 70 or 80°F. in 15 minutes. Agitation is usual during cooling, followed by more violent agitation for about half a minute. Sterilisation usually produces a soft junket-like curd which shakes down to a homogeneous liquid. In order to detect faulty tins and infected samples, the tins are incubated at 80–97°F. for periods varying from 10 to 30 days.

Heat coagulation of casein is influenced by time and temperature of sterilisation, concentration, forewarming, factors which

[1] Hunziker, 1935, *Condensed Milk and Milk Powder*, p. 224.

come under the control of the manufacturer; and others which are essentially determined by the raw milk. Among the latter are developed lactic acid, albumin content, salt balance and contamination with proteolytic bacteria. One of these, the salt balance merits some further attention. Milk calcium is normally balanced between phosphoric and citric acids on the one hand and casein on the other. To a lesser degree magnesium is also concerned in this equilibrium. If the two bases are increased, or if the ratio is disturbed by increases of the two acids, it is reasonable to suppose that the quantity of calcium held by casein will be greater or less than normal. Hunziker[1] presents the known facts and suggests that casein is rendered heat unstable by such abnormalities in attached calcium. The more common manifestation of a disturbed salt balance is an increase in the ratio Ca+Mg/citrate+phosphate, which would suggest that in such circumstances addition of sodium citrate or phosphate would render the milk heat stable.

This is the basis of a process of "pilot sterilisation" in which various quantities of the selected stabiliser are added to the evaporated milk in 6 ounce tins, which are then sterilised, and the condition of the product carefully examined in order to determine whether any stabiliser is necessary, and if so in what amount it should be used. Sommer and Hart[2] found that 2 to 10 ounces of stabiliser per 1,000 lbs. of evaporated milk were usually sufficient. As the efficiency of the stabiliser is greater when added prior to forewarming, the results of one day's pilot trials may be applied to the next day's condensing, the correct quantity being added before forewarming.

Some Faults of Condensed Milk.—In sweetened condensed milk, a common fault is grittiness, the cause of which has been considered earlier in this section. Another defect of this product is a progressive thickening which may be of bacterial or physico-chemical origin. Storage temperatures below 45°F. are very successful in reducing this change to a minimum. Above this temperature, the process is progressive. As already pointed out, the temperature of forewarming also determines to some extent the course of this change. Blown tins are due to contamination with yeast cells which may enter the milk from the atmosphere or through the use of damp sugar. The sucrose of sweetened

[1] Hunziker, 1935, *Condensed Milk and Milk Powder*, p. 248.
[2] Sommer and Hart, 1926, *Wis. Res. Bull.*, 67.

condensed milk sometimes suffers hydrolysis to give fructose (laevulose) and glucose. The former may undergo a process of condensation giving a gum-like substance named laevulan. A sample examined in 1929 showed that 6 per cent of the total sucrose had been altered in this manner. Three years later the change had affected 11–12 per cent of the total. This change does not necessarily spoil the product, but it introduces certain complications in the estimation of sucrose in samples of sweetened condensed milk which have been kept for a considerable length of time.[1] It is interesting to note that lactose does not suffer a similar change. The hydrolysis of lactose would produce glucose and galactose, but not fructose.

Curdiness may appear in unsweetened condensed milk through heat coagulation of casein. A grainy sediment has been observed on occasion, which has proved to consist of calcium citrate and phosphate rendered insoluble by the action of heat.

Buttermilk and whey may be condensed. The former makes a valuable animal feeding-stuff, and the latter may be used as such or for the preparation of lactose.

The Composition of Condensed Milk and Milk Products.—Whole milk of 3·6 per cent and 8·8 per cent fat non-fatty solids must be condensed to $\frac{1}{2{\cdot}5}$ part of its original bulk, in order to give a product containing the minima of 9 per cent fat and 22 per cent non-fatty solids. It follows that the percentage of each individual constituent in milk is multiplied by 2·5 to give the percentage in the final product as illustrated in the following table:

Composition of the milk %	Calculated composition of:	
	Unsweetened condensed milk	Sweetened condensed milk
Fat 3·6	3·6 × 2·5 = 9·0% minimum	9·0% minimum
Lactose 4·7	4·7 × 2·5 = 11·75 ,,	11·75 ,,
Protein 3·35	3·35 × 2·5 = 8·38 ,,	8·38 ,,
Ash 0·75	0·75 × 2·5 = 1·87 ,,	1·87 ,,
		added sucrose 17 × 2·5 = 42·5
Water 87·6	by diff. 69·0	26·5
100·0	100·0	100·0

[1] *Analyst*, 1932, 57, 630.

Actual analyses given by Cox[1] are as follows:

	Condensed Full-cream Milk		Condensed Separated Milk Sweetened
	Unsweetened	Sweetened	
Fat	9·10%	9·60%	0·29%
Lactose	12·74	13·07	14·25
Protein	8·75	8·41	9·53
Ash	1·94	1·86	2·31
Sucrose	—	41·92	46·59
Water	67·47	25·14	27·03
	100·00	100·00	100·00

Milk Powders.—Early attempts to reduce milk to a powdered form were hampered by loss of solubility occasioned by changes in casein during the process of drying. To counteract this tendency, it became necessary to add alkali carbonates to the milk before drying. Grimwade, to whom was granted a British patent in 1855, added an alkali carbonate to milk which he evaporated with constant agitation to a dough-like consistency in an open-jacketed evaporating pan. Sugar was then added and the dough pressed into ribbons by passing between rollers. The strips were then dried further, and the product finally ground to a powder.

The modern processes, by shortening the time of heating, have successfully overcome this difficulty, and it is rarely necessary to add a stabiliser. Many processes are in operation, but the most common fall into two groups: Roller, Drum, or Film drying, and the Spray or Atomiser processes.

All of the former methods depend upon the distribution of milk in the form of a thin film over the surface of revolving rollers heated internally by steam. By the time the film is dry, it is scraped from the surface by means of a knife or scraper, which must be accurately placed so that the film is neither incompletely dry nor overheated. The milk thus removed is in the form of a flaky film which must be ground to a powder before packing. The milk may or may not be pre-condensed.

If not condensed, the common practice is to feed the liquid into the upper '⌣'-shaped cavity between two rollers rotating towards each other with a gap of about 0·02 inch between them.

[1] Cox, 1938, *The Chemical Analysis of Foods*, p. 257.

The heat of the cylinder keeps the milk hot before it passes through the gap to be taken up as a film by each roller.

If the liquid has been pre-condensed, it may be taken up by the heated roller which dips into it; or a small unheated roller may dip into the condensed milk, transfering the film to the heated one which revolves in contact with the smaller. Only by pre-condensing is it possible to obtain a sufficiently thick film by this method of "pick up."

The thickness and uniformity of the film are very important factors. If too thin, drying is very rapid and overheating is liable to occur. If too thick, the drying may not be complete. Uniformity of thickness is also desirable, although difficult to secure. If the steam pressure is increased, drying is more rapid and the speed of rotation of the roller must be increased in order to avoid over-heating. Thus it would seem that increase of steam-pressure leads to increased output per roller. However, other factors are involved, and this relationship holds good only within a restricted range. A short period of heating is essential if the solubility of the powder is not to suffer severe losses. The whole process may be carried out at atmospheric pressure under a hood to remove the steam, or the drying may take place at a much lower temperature under reduced pressure.

The precise combination of these variants depends very much upon the material to be dried, the purpose for which the dried product is intended, etc. In one of the double roller machines the rollers measure 10 ft. long by 3 ft. diameter, and they make no more than 16 revolutions per minute, the steam pressure varying from 48 to 80 lbs. per square inch.

The spray or atomiser drying process depends upon converting the liquid into an exceedingly fine mist in which the droplets are about 0·002 inch in diameter, and which are caused to evaporate spontaneously by contact with a current of hot air. There are various methods of obtaining the spray. The liquid may be forced at 2,000 to 3,000 lbs. pressure through nozzles which may be stationary or rotary. Another method is to allow the liquid to fall on a rapidly revolving disc which flings it away by centrifugal force as a fine spray. The principle of the scent spray is also used in which a jet of the liquid meets a powerful blast of compressed air. Pre-condensing is usual, one reason being that heat is used more efficiently in the vacuum condenser than in the spray chamber. The hot air may be between 100 and 150°F.,

and operational details vary so much that only the underlying principles are given in this treatment of the subject. Those interested in such technical details should consult Hunziker's monumental work on the subject. Evaporation is practically instantaneous, and a fine dust of dried milk settles to the floor of the drying chamber, from which it is removed mechanically, and without further treatment the powder is packed. Recovery of fine dust particles carried away by the outgoing air is effected by dust filters or by the simple expedient of washing the outgoing air with a spray of incoming milk.

Composition of Dried Milk and some Dried Milk Products.—The moisture content of milk powders depends upon the method of preparation and conditions of storage. It is useful to remember that 2·5 per cent and 5 per cent are fair averages for the moisture content of spray and roller dried milk respectively. The solid matter being 97·5 or 95 per cent, it follows that the concentration factor for normal milk is slightly less than 8 and that in dried whole milk the percentage of any milk constituent is slightly less than eight times the normal for liquid milk. By the same reasoning in dried separated milk the concentration factor is about 10·5 and for dried whey it amounts to approximately 14. The dried milk regulations 1923 and 1927 provide standards of composition for various grades of dried milk, in addition to dried separated milk. In the latter, the container must bear the words "not to be used for babies," not because the product is at all harmful, but because the nutritive value of separated milk is so much lower than that of milk that there must be no confusion in the mind of the purchaser arising from the designation "milk" in both products. In all cases, the number of pints of reconstituted milk equivalent to the contents of the package must be stated clearly.

DRIED MILK REGULATIONS

Dried milk to contain at least the following % Fat:		Milk from which the powder was made has the composition:	
Full cream milk	26	3·6% fat	8·8% non-fatty solids
¾ ,, ,,	20	2·7 ,,	8·9 ,, ,,
½ ,, ,,	14	1·8 ,,	9·0 ,, ,,
¼ ,, ,,	8	0·9 ,,	9·0 ,, ,,
Separated	—		9·0 ,, ,,

The composition of some dried products is shown in the following table.

THE COMPOSITION OF DRIED MILK AND DRIED MILK PRODUCTS[1]

	Water %	Fat %	Protein %	Lactose %	Lactic acid %	Ash %	Maltose + Dextrose %	Fibre %
Whole milk	2·5	27·5	26·5	37·5	—	6·0	—	—
Separated milk	2·5	1·0	38·0	50·5	—	8·0	—	—
Cream	0·7	65·0	13·4	17·9	—	3·0	—	—
Buttermilk	7·0	6·5	36·5	35·5	6·0	8·5	—	—
Whey	4·0	5·0	12·0	70·0	—	9·0	—	—
Malted milk	3·0	8·0	18·0	12·0	—	3·8	55·0	0·2

Elsdon and Walker[2] quote figures for the analysis of dried milk to which sucrose has been added, the concentration in the dried product being approximately 2 per cent.

Some Properties of Milk Powder.—If dried milk is intended for human consumption, it must 'dissolve' in water, leaving no sediment, whereas if intended for animal feeding, complete solubility is not a matter of great importance. The following factors are known to lower the solubility of milk powders: high acidity before drying, high temperature or prolonged heating, high moisture content of the powder, ageing of the sample, roller drying under atmospheric pressure as compared with spray drying. Casein and mineral matter appear to be chiefly concerned in this respect. A good quality sweet milk dried by the spray process and stored in air-tight moisture-proof packages is practically completely soluble, provided that the milk has not been heated above 150°F. prior to drying. Atmospheric roller drying gives a product which is usually less soluble. Hunziker[3] gives the following conditions of overheating which cause a 50 per cent loss of solubility of the casein: 1 second at 160°C., 4 seconds at 155°C., 7 seconds at 150°C., 10 seconds at 145°C., and 40 seconds at 139°C. Both roller and spray types become less soluble with age, but the change is much more rapid with increasing moisture content and storage temperatures. The precise change in casein rendering it insoluble by reason of moisture absorption by the powder is rather obscure, but the important practical consideration is that the containers must be thoroughly air- and moisture-proof.

[1] Hunziker, *Dried Milk and Dried Milk Products*, 1928, Douthitt Eng. Co., p. 29
[2] Elsdon and Walker, 1941, Richmond's *Dairy Chemistry*, p. 162.
[3] Hunziker, 1935, *Condensed Milk and Milk Powder*, p. 535.

One other interesting point of difference between roller-dried and spray-dried milk is that in the former, lactose is present as the crystalline hydrate, whereas in the latter it forms an anhydrous film which protects the fat globules from the action of fat solvents. Addition of water to spray powders renders the fat more vulnerable to solvent action.[1]

The Development of a Tallowy Taint.—Milk powders are in a very fine state of division and, when exposed to the air, offer a large surface area, thus facilitating oxidative changes which lead to the production of tallowiness. The general experience has been that the spray powders are more prone to this defect than are roller-dried samples. There may be several causes, but one factor is undoubtedly the difference in physical condition of these two types. Spray powders are exceedingly fine, being only about 0·002 inch in diameter, while the roller-dried material is usually ground to about 0·02 inch. Again, the former grains are porous and contain a central air bubble whereas the latter are flaky and more compact. All of these differences facilitate oxidation of the spray type rather than the roller powders. Light, heat, acidity, and contamination with metals, especially copper, all hasten the oxidative changes.

Much attention has been given to the effect of moisture content of the powders on the development of tallowiness, but the evidence is contradictory and the whole problem requires further elucidation. Acidity and traces of copper exert the influence which might be expected of them in their encouragement of oxidation. Supplee[2] found that milk contaminated with copper to the extent of 3 to 7 parts of copper per million of milk solids, gave a powder which developed a tallowy taint in five months. Normal uncontaminated milk powder required over twelve months for the taint to appear. Iron is less efficacious than copper in this respect, but milk is able to dissolve much more iron than copper, and on this account it is desirable to avoid contamination with this metal so far as possible.

Packing must ensure that air does not gain access, not merely because of the possibility of oxidation, but because the powders are so very hygroscopic. Supplee[3] exposed powders to atmospheres of increasing humidity and found that they reached equilibrium with the atmosphere with the result that their

[1] Lampitt and Bushill, 1931, *J. Soc. Chem. Ind.*, 50, 45.
[2] Supplee, 1923, *Proc. World's Dairy Congress, Washington.*
[3] Ibid.

moisture content increased as the air became more humid. At a humidity of 20 to 30 per cent the powders contained 3·5 to 4·5 per cent moisture. Increasing the humidity to 50 to 60 per cent and 70 to 80 per cent, increased the moisture to 6 to 7 per cent and 9 to 12 per cent respectively. Moisture absorption causes a deterioration of flavour in addition to reducing casein solubility.

It has been found possible to reduce or eliminate oxidative changes by three expedients[1]:—

(*a*) Compression of the powders into cubical blocks weighing about 30 lbs.,

(*b*) Packing the powder in an atmosphere of nitrogen,

(*c*) Pasteurisation of the milk at 190°F instead of 165°F.

The first would seem admirable for treatment of powders intended for long distance transport. At the time of writing, all of these developments are in their preliminary stages, but there is good reason to hope that they will lead to a satisfactory solution of the problem.

[1] Quoted by Hilditch, 1944, *Chem. and Ind.*, p. 67.

CHAPTER VIII

DAIRY BY-PRODUCTS AND MISCELLANEOUS SUBJECTS

The chief dairy by-products, whey, separated milk, and buttermilk, are valuable foods, but their perishable nature necessitates rapid utilisation; or, if this is not possible, they must be condensed, dried, or otherwise treated. Their virtues as animal feeding-stuffs are well established and in the liquid form large quantities are utilised in this manner. There are, however, serious limitations to this procedure. When butter and cheese were more generally farmhouse products, these by-products were produced under circumstances which enabled their immediate local utilisation, but with the advent of the creamery and cheese factory large surplus quantities of the by-products accumulated. Under these conditions there is a tendency to regard them as perishable goods to be disposed of rather than valuable foods to be utilised to fullest advantage. Further, it is the conviction of many that these by-products are utilised to the full only when used for human nutrition. We are reminded of whey by Samuel Pepys[1] in his own inimitable style: "June 10, 1663. Thence to the whey house and drank a great deal of whey, and so by water home." It is certainly significant that in those days of large and unrestrained appetites, there was a place for liquid whey in the diet of such a joyous gourmand as Pepys. The ancients were familiar with its medicinal properties, for Hippocrates prescribed whey for the cure of scurvy, intestinal and even mental disorders. There is no doubt of the historical popularity of whey. These considerations give cause for some anxiety over the present-day neglect of this commodity as a human food.

The table on page 185 gives the composition of typical samples of these by-products which, naturally, show some variation.

Utilisation of the three by-products in their complete forms as feeding-stuffs may be effected in the raw, condensed, or dried conditions. Condensation and drying, besides rendering them less perishable, reduce transport costs. They have been used very successfully in the baking and confectionery industries. When

[1] *The Diary of Samuel Pepys*, entry for June 10, 1663.

PERCENTAGE COMPOSITION OF WHEY, SEPARATED MILK, AND BUTTERMILK

	Whey	Separated Milk	Buttermilk
Water	93·4	90·6	90·8
Fat	0·3	0·1	0·5
Lactose	4·8	4·9	3·9
Lactic acid	undetermined	undetermined	0·6
Protein	0·9	3·6	3·5
Ash	0·6	0·8	0·7
Vitamins	All water soluble vitamins of milk present Fat soluble vitamins negligible		

water is replaced by these by-products the yield of bread per 100 lb. of flour is increased by the following amounts: 10–11 lb. by separated milk, 5–6 lb. by whey, 12–13 lb. by whey condensed to half its bulk, and 9–10 lb. by buttermilk. Quality as well as yield were improved.

Other methods of utilisation are shown in the table on page 190. There are many possible methods of whey utilisation, but market conditions determine very largely whether they can be put into operation. For instance, the obvious treatment is to condense and separate lactose crystals. Only a fraction of the lactose is recovered because the high costs of more complete recovery would render the process uneconomic. It is therefore more profitable to effect a partial removal of lactose and to combine the mother liquors with a suitable vegetable feeding-stuff, or to condense them further to give whey paste.

Two methods may be adopted. After separation of whey cream which may be churned to butter, the whey may be condensed in a vacuum evaporator to about 60 per cent total solids. From this syrup lactose is allowed to crystallise slowly. The crude lactose may be sold as such or may be further purified by crystallisation from water. The mother liquor, still containing appreciable quantities of lactose, protein, and mineral matter, may be condensed to whey paste or united with a suitable animal feeding-stuff. The whey paste contains about 50 per cent total solids. Both are valuable animal feeding-stuffs. In the other method, the whey after separation is neutralised with lime and heated to coagulate

albumin. This is allowed to settle and the supernatant liquid decanted, condensed, and allowed to crystallise. Lactose crystals are removed and the mother liquor is combined with the dried albumin obtained earlier in the process. The mixture is dried and forms a valuable food which has gained popularity amongst poultry farmers. The heating of whey to produce coagulated albumin intended for human consumption as 'fleetings' or 'primost' is a very old custom. A variation of this process was introduced by the West of Scotland Agricultural College. After heating and allowing the albumin to settle, six-sevenths of the supernatant liquid is drawn off, and the residue is homogenised to give a liquid which has the appearance of milk. The composition of this whey milk is: fat 0·5–2·5 per cent, protein 3·5–4·5 per cent, lactose 4·5–5·0 per cent, ash 0·45–0·55 per cent, water 87·5–90 per cent. It has found several uses as an ingredient of ice-cream mixes, in the confectionery trade, and as a substitute for buttermilk in bakery. Although whey may be fermented to lactic acid, butyric acid, or to alcohol and acetic acid, the commercial success of such operations depends upon many factors which cannot be discussed fully in a book of this description. The same remarks would also apply to any venture to extract riboflavin from whey.

If not used as a food, separated milk is used for the preparation of rennet casein and acid caseins, which, as already described (page 38), are raw materials for the production of a large variety of products such as plastics, glues, cements, paints, medicinal preparations, patent foods, etc. The filtrates may be treated by any of the methods applicable to whey. Buttermilk is not used for this purpose so much as separated milk.

Water Supply for Dairy Purposes.—It is highly desirable that water used in dairying should be uncontaminated with harmful bacteria, for even although churns and milk bottles may be steam sterilised, and although it may be claimed that milk never has direct contact with such water, experience has proved that such a supply is a constant menace. Several faults of dairy produce have been attributed to the water supply. While these claims are justified by experience, it is not always realised that dairymen are rarely able to choose their water supply and that milk is sometimes produced under conditions in which it is difficult at times to find enough water to run through the cooler. Even so, much can be done by frequent attention to wells, drainage from

the farmhouse or buildings, etc., in order to eliminate the possibilities of sewage contamination. If the water supply is known to be contaminated every effort should be made to discover and eliminate the source of the trouble, but in the meantime, it may be necessary to utilise chlorination. This step should be taken only on the advice of a bacteriologist qualified to deal with such matters. Frequent checking of the efficiency of the measures taken would be necessary.

From a chemical point of view the most practical consideration is the hardness of the water supply. Boiler scale, or scale formation in bottle-washing machines are the result of hardness in the water used for these purposes. Calcium bicarbonate dissolved in water is precipitated as calcium carbonate when the water is boiled, or with an alkali detergent a film of calcium carbonate settles on the parts of washing machines which are in contact with the detergent. The chemical changes are represented by the equations—

$$\xrightarrow{\text{boil}} \quad Ca(HCO_3)_2 = CaCO_3 + CO_2 + H_2O$$

$$Ca(HCO_3)_2 + 2\,NaOH = CaCO_3 + Na_2CO_3 + 2\,H_2O$$

Because this type of hardness is removed by boiling it is called temporary hardness in order to distinguish it from permanent hardness, which is due to the presence of dissolved calcium sulphate or magnesium sulphate, neither of which suffers any change when the water is heated to boiling. When used for raising steam, the solution becomes more and more concentrated, and as calcium sulphate is only sparingly soluble, it forms a very hard crystalline boiler scale which is much more difficult to remove than the calcium carbonate formed by temporarily hard water. The student is warned not to misinterpret the term 'temporary' as meaning a water which will not cause trouble when used in a boiler. Both types cause a great deal of trouble, the only difference being that the scale from one is somewhat less tenacious than that from the other. Similarly, both types cause scale when in contact with alkaline detergents. A permanently hard water will react as follows—

$$CaSO_4 + Na_2CO_3 = CaCO_3\downarrow + Na_2SO_4$$

$$MgSO_4 + 2\,NaOH = Mg(OH)_2\downarrow + Na_2SO_4$$

Very few natural water supplies are free from hardness and the question often arises whether it is desirable to install a water softener to avoid constant de-scaling and wear and tear of the

boiler. The problem does not lend itself to hard and fast recommendations. Much depends on the degree of hardness of the water supply and the steam requirements of the dairy.

Common methods of water softening are the lime-soda process and the zeolite or base exchange system. In the former, the calculated quantities of hydrated lime $(CaOH)_2$ and sodium carbonate Na_2CO_3 are added in order to precipitate the compounds responsible for temporary and permanent hardness. The zeolite process involves the percolation of water through a vessel containing the zeolite whereupon a process of base exchange is brought about, calcium and magnesium leaving the water to enter the zeolite and their places being taken by sodium.

$$\text{Na-zeolite} + \text{Ca-water} = \text{Ca-zeolite} + \text{Na-water}$$

By this method both types of hardness are removed, and the process is self-regulating. The zeolite may be recharged by running a solution of salt through the apparatus.

$$\text{Ca-zeolite} + NaCl = \text{Na-zeolite} + \text{Calcium chloride}$$

For convenience, the zeolite process has much to commend it.

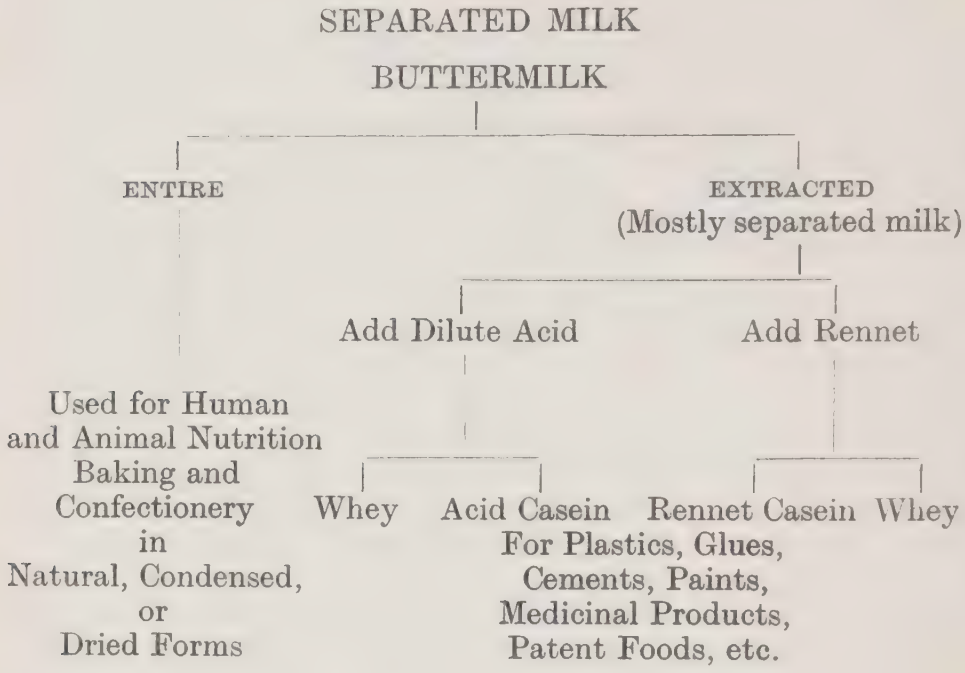
Salt for Dairy Purposes.—Salt is obtained in this country from several natural salt deposits in Cheshire and Worcestershire. The brines from which the salt is crystallised may contain some 25 per cent sodium chloride, 0·2 per cent magnesium chloride, 0·4 per cent calcium sulphate, and a trace of calcium bicarbonate. The operations of brine purification, evaporation, and crystallisation have as their object the elimination of these impurities from the salt. Calcium sulphate being only slightly soluble may impart a gritty texture to the salted dairy product while magnesium chloride, being intensely hygroscopic, tends to make the salt damp. Contrary to the general belief in the germicidal properties of salt, it is known that damp salt may introduce various micro-organisms into dairy products. Only by keeping salt dry is it possible to avoid such contamination. As it leaves the factory, salt is usually in a very pure condition, but when allowed to become moist in the dairy or when stored in sacks which have been used for other purposes, contamination is likely to occur. For dairy purposes, it is also desirable that the salt should be readily soluble, and ease of solution depends upon its fineness. It is desirable that the salt should pass a 10-mesh sieve completely and that 90 per cent of it should pass through an 18-mesh.

The British Standards Institution have issued specifications for dairy salt in which it is stated that no more than 0·2 per cent moisture should be present and that calcium or magnesium should not exceed 0·01 per cent. The designation 'Vacuum salt' refers to the improved purity of salt as prepared by vacuum evaporation as opposed to the older open-pan evaporators.

The British Standards Institution.—One of the many achievements of the Empire Marketing Board was the establishment of a Dairy Research Committee, whose activities included an examination of methods of analysis of dairy products and of materials and apparatus used in such tests. When the Empire Marketing Board came to an end in 1933, the work of this committee was transferred to the British Standards Institution, which assists many industries by standardisation and improvement of methods used in the examination of their products or raw materials. Specifications of special apparatus, glassware, etc., are issued from time to time for the guidance of manufacturers of these goods.

Several reports of interest to dairy technologists and others have been issued by the publications department, 28, Victoria Street, London, S.W.1. They include: the sampling of dairy products, the chemical analysis of butter and cheese, fat estimation by the Gerber and Babcock methods, salt for dairy purposes, vegetable parchment for the wrapping of dairy products, density hydrometers for use with milk, floating dairy thermometers, etc., all of which are moderately priced. Apparatus which has been made to these specifications bears the mark 'B.S.S.', which is some guarantee that the accuracy is of a sufficiently high standard for ordinary purposes.

Sometimes thermometers and other glassware bear the inscription 'N.P.,' which means that a certificate of accuracy has been issued for that particular instrument by the National Physical Laboratory. Apparatus so marked is of a greater degree of accuracy, there being two grades: Class 'A' for work of highest accuracy, and Class 'B' for general commercial requirements.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BUTTERMILK
ENTIRE
EXTRACTED
(Mostly separated milk)
Add Dilute Acid
Add Rennet
Used for Human and Animal Nutrition Baking and Confectionery in Natural, Condensed, or Dried Forms
Whey
Acid Casein
For Plastics, Glues, Cements, Paints, Medicinal Products, Patent Foods, etc.
Rennet Casein
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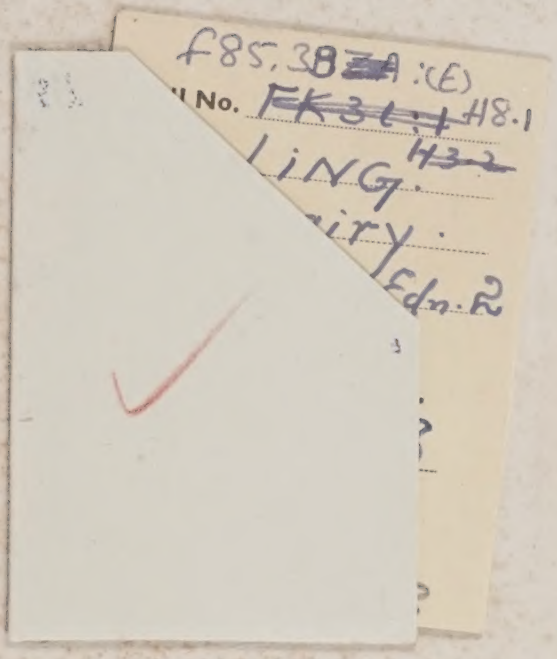
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